MERCK

User Guide

Auto2D®
Two Dimensional Electrophoresis Device

BM-100

SigmaAldrich.com

Millipore®

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Auto2D® Device

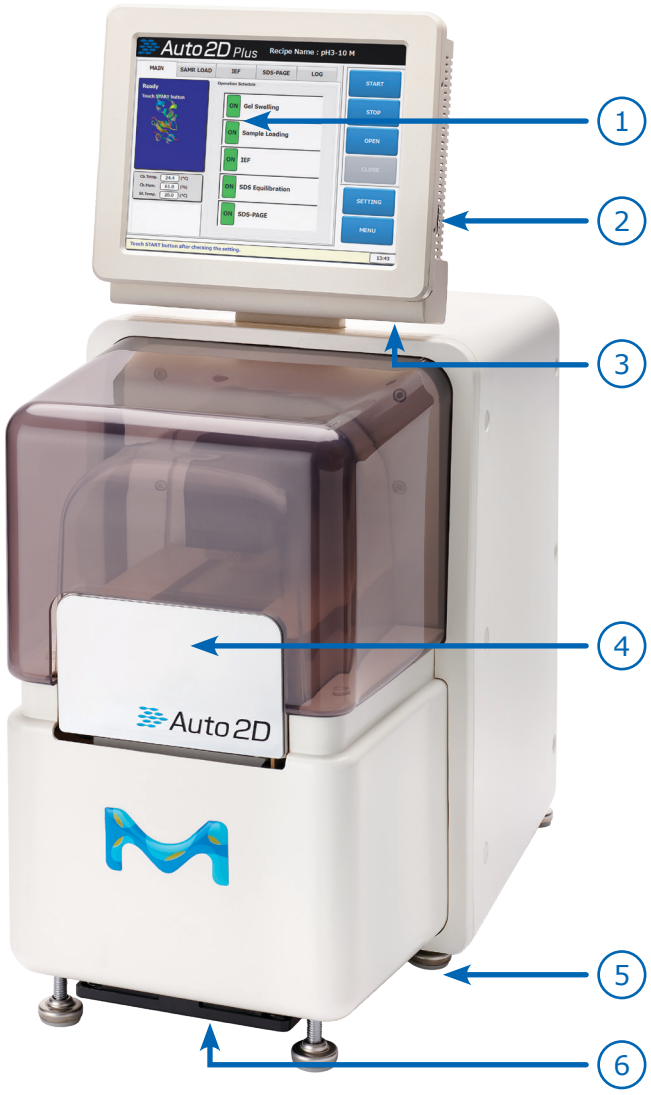
Intended Use

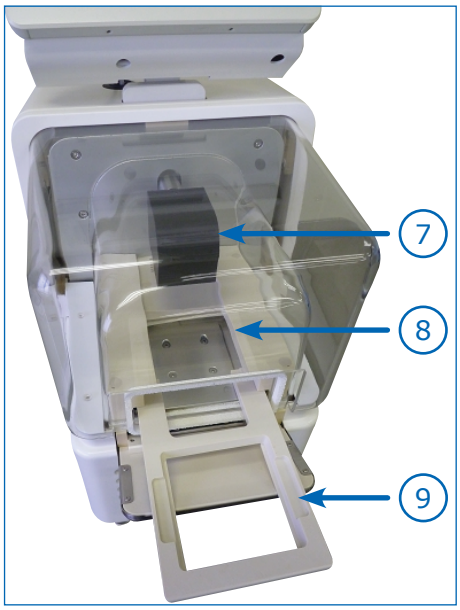
The Auto2D® 2-D Electrophoresis Device (Auto2D® Device) automatically separates thousands of proteins in a short period of time utilizing their specific isoelectric point differences (First Dimension) and their molecular weight (Second Dimension).

Before using this device, please read the Safety Sheet available in the product box and online at SigmaAldrich.com on the Auto2D® product pages.

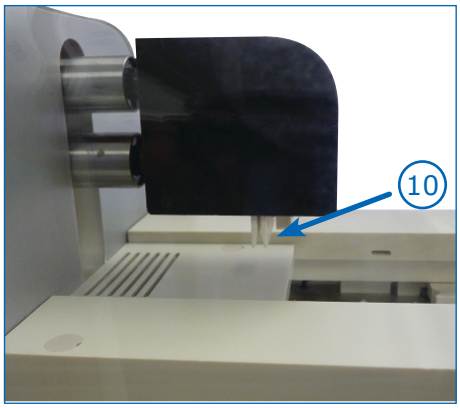
For research use only.

Auto2D® Device Features

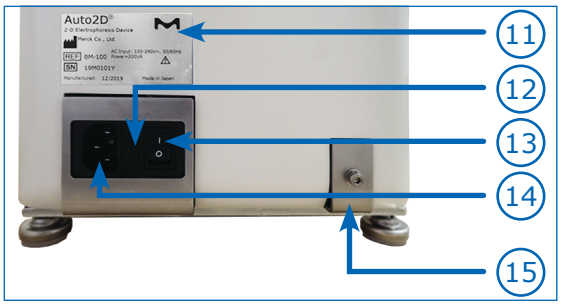




Clamp View



Rear View



Front View

1. Control Panel (LCD Touch Panel)
   Operate the device through this panel
2. USB Ports Output Data
3. Power Reset (below screen)
4. Tray Cover for the Chip Assembly Tray
5. Height Adjuster x 6
   Levels the Auto2D® Device on a table
6. Fan Filter

Chamber View

7. Arm
8. Electrophoresis Chamber
9. Chip Assembly Tray

IEF Chip View

10. Clamp for the IEF Chip

Rear View

11. Rating Label (Specifies Input Voltage)
12. Fuse Holder
13. Power Switch
14. AC Inlet
15. Equipotential Bonding Terminal Arm (an electrical connection maintaining various exposed conductive parts and extraneous conductive parts at the same potential)

Specifications

Main Body

Dimensions width x depth x height	240 mm × 427 mm × 530 mm (9.45 in. x 16.81 in. x 20.87 in.)
Weight	Approximately 17 kg (37.48 lbs)

Control Panel (Panel Computer)

Screen Size	230.2 mm (8 in.) screen with touch panel function
Resolution	800 × 600 SVGA
Memory Capacity	DDR3L (4 GB) RAM,
SSD	128 GB built in SSD
I/O	USB 2.0 (Two ports)

Electrophoresis

First Dimension

Voltage Range	DC, 50–10,000 V
Voltage Application	7 steps (linear up/down, maintain)
Maximum Output	2 W

Second Dimension

Voltage Range	DC, 10–400 V
Maximum Output	20 W
Control Method	Constant current control by software Constant voltage and constant power controls are also available

Safety Sheet

Additional specifications and information can be found in the Safety Sheet. A printed copy of the Safety Sheet has been included in the product box and is also downloadable from the Safety and Documentation section on the product page at SigmaAldrich.com. This document includes:

- Electrical Specifications
- Operating Conditions
- Storage and Stability
- Warnings
- End of Life Instructions WEEE Directive
- Symbol Definitions

Additional Information

Included with Auto2D® Device

- 1 Auto2D® Electrophoresis Device
- Quick Start Guides and Safety Sheet
- Electrical Power Cables:
  - Power cable for JP and North America
  - 3P-2P Conversion Plug for JP and North America
  - Power cable for Europe
  - Power cable for China
  - Power cable for UK

Also Needed (not included)

See *Product Ordering on page 54* for catalogue numbers.

- Auto2D® Electrode Chip Plus or Auto2D® Electrode Chip (original)
- Auto2D® PAGE Chip
- Auto2D® IEF Chip
- Auto2D® Solution Chip Plus or Auto2D® Solution Chip (original)
- Auto2D® Tris-Glycine or Tris-Tricine Reagent Kit
- Ampholyte (choose appropriate ampholyte based on IEF Chip pH range)
- Distilled water
- Filter paper 0.3 mm thick (optional)

Consumables

Electrode Chip and Electrode Chip Plus

- Life span of 100 uses.
- Only for use with Auto2D® Device.
- For research use only, not for clinical use.

Specifications

Materials of Construction

Body	PPS
Electrode	PT
Electrical Connection	Gold-plated beryllium copper

Stability

Operating Temperature	< 40 °C

If you heat to dry, do not exceed 40 °C.
Keep away from direct sunlight and high temperature.
Store in case provided.

Electrode Chip Care

- Immediately after use, rinse with distilled water and allow to air dry.
- After use, do not heat above 40 °C while drying. Doing so will risk deformation or deterioration. Do not use when wet.
- If there are many deposits on the electrodes, etc., contact Technical Service at SigmaAldrich.com/techservice
- If you are conducting continuous analysis, we recommend that you purchase additional Electrode Chips.

CAUTION: If Electrode Chip platinum wire is cut, it will be a stabbing hazard and may cut you. Discontinue use and contact technical service at SigmaAldrich.com/techservice for additional help.

PAGE Chip

- Only use with Auto2D® Device.
- Do not store in vertical position.

Specifications

Materials of Construction

Main Component	Polyacrylamide
Case	PMMA (low fluorescence type)

Stability

Lot Number	See packaging label
Expiration Date	See packaging label
Storage Temperature	2 °C to 8 °C

Do not place near the cold air outlet of the refrigerator.
There is a risk of freezing.

Gel Concentration (T%)	Qty	Catalogue Number
6.5%	10	BM-12065
7.5%	10	BM-12075
10.0%	10	BM-12100
12.5%	10	BM-12125

Gel Size	60 mm x 50 mm (2.36 in. x 1.97 in.)

IEF Chip

Only use with Auto2D® Device.

Specifications

Materials of Construction

Main Component	Polyacrylamide
Support Plate	PMMA

Stability

Lot Number	See packaging label
Expiration Date	See packaging label
Storage Temperature	-20 °C
IPG Gel Length	50 mm (1.97 in.)

pH Range	Qty	Catalogue Number
3–10	10	BM-113010
3–10 NL (Non-Linear)	10	BM-113010NL
4–7	10	BM-114070
4–5.5	10	BM-114055
5–6.5	10	BM-115065
6–10	10	BM-116010
7–10	10	BM-117010

Solution Chip and Solution Chip Plus

- Single use only
- Only use with Auto2D® Device.

Specifications

Materials of Construction

Body	PMMA

Stability

Storage Temperature	Room Temperature

Keep away from direct sunlight and high temperature. Use included case for storage.

Tris-Glycine Reagent Kit

Kit Contents

- Rehydration Solution Kit (7.7 g powder, 10 mL volume after preparation) with 52 dispensing tubes
- DTT Kit (0.54 g powder, 3.5 mL volume after preparation) with 52 dispensing tubes
- Tris-Glycine Equilibration Buffer Premix (50 mL solution)
- Anode Buffer (250 mL solution)
- Tris-Glycine Cathode Buffer (250 mL solution)

Also Needed (not included)

- Distilled water for preparation.
- Ampholyte (choose appropriate ampholyte based on IEF Chip pH range)

Storage and Stability

Rehydration Solution Kit should be stored at room temperature before reconstituting powder. The remaining components should be stored at 2–8 °C. Use the unopened kit within one year from the production date indicated on the label.

Tris-Tricine Reagent Kit

Kit Contents

- Rehydration Solution Kit (7.7 g powder, 10 mL volume after preparation) with 52 dispensing tubes
- DTT Kit (0.54 g powder, 3.5 mL volume after preparation) with 52 dispensing tubes
- Tris-Tricine Equilibration Buffer Premix (50 mL solution)
- Anode Buffer (250 mL solution)
- Tris-Tricine Cathode Buffer (250 mL solution)

Also Needed (not included)

- Distilled water for preparation.
- Ampholyte (choose appropriate ampholyte based on IEF Chip pH range)

Storage and Stability

Rehydration Solution Kit should be stored at room temperature before reconstituting powder. The remaining components should be stored at 2–8 °C. Use the unopened kit within one year from the production date indicated on the label.

Auto2D® Device Installation

Location

Install and use the Auto2D® Device in a location not exposed to:

- Direct sunlight and heat
- Magnets or devices that generate a magnetic field
- High humidity
- Mechanical vibration or impact

Place the Auto2D® Device on a level surface.

When moving the Auto2D® Device, be careful to lift from the bottom of the back sides. Do not lift from the bottom of the front to avoid damaging the fan filter.

Compatible USB Devices

Two USB ports are available for USB flash drive connection. Auto2D® Device does not support other connections (serial port, etc.) or devices.

ATTENTION: Be sure to run anti-virus software on the USB flash drive before using with Auto2D® Device. Never connect a device other than a USB flash drive. Auto2D® Device does not support internet connection.

Connecting Power

Select the appropriate power cord (provided with the device) that is compatible with the power outlets for your location. Firmly push the matching end into the back of the Auto2D® Device, and the other end into the power outlet.

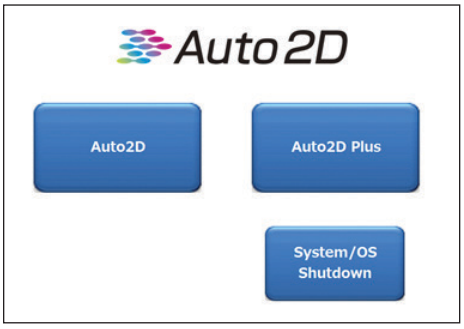
The following power cables are shipped with the Auto2D® Device:

- Electrical Power Cables:
  - Power cable for JP and North America
  - 3P-2P Conversion Plug for JP and North America
  - Power cable for Europe
  - Power cable for China
  - Power cable for UK

Device Operation

How to Turn On

The power switch is located next to the AC plug port on the back of the Auto2D® Device. Press switch up to turn on. The LCD touchscreen will display two operations. Detailed directions for *Auto2D® Plus Mode on page 9,* and *Auto2D® Mode (Original) on page 34.*



How to Turn Off

Touch [MENU] > [EXIT APPLICATION] > [OK] > [SYSTEM/OS SHUTDOWN] > [OK] . Once complete, turn the power switch (located on back of device) to the "Off" position.

NOTE: If there is condensation inside the device, leave tray open until the inside dries. When the device is dry, close the tray and turn the power off.

CAUTION: In rare cases, the system will not boot up properly if not turned off correctly. Please turn off according to the instructions to avoid persistent issues or damage to the Auto2D® Device. If the main power is on but the system does not start up, a reset switch is located on the lower right of the touch panel (arrow 3 on *page 4*).

Auto2D® Plus Mode

The Auto2D® Plus mode is the improved operation mode for Auto2D® electrophoresis. Plus Mode actively loads proteins into rehydrated gel by applying voltage. Compared with Auto2D® mode (Original), which adopted conventional electrophoresis method, the sample loading efficiency is very high and the run time is 30 minutes shorter.

More spots can be detected in Auto2D® Plus mode because a higher protein amount can be applied, and protein absorption rate into gel is higher than Auto2D® mode (Original). Also, the Auto2D® Plus mode offers new features to simplify sample preparation: Desalt recipes and Auto-Stain recipes.

Auto2D® Plus mode is highly recommended unless you need to keep consistency with past data obtained by Auto2D® mode (Original).

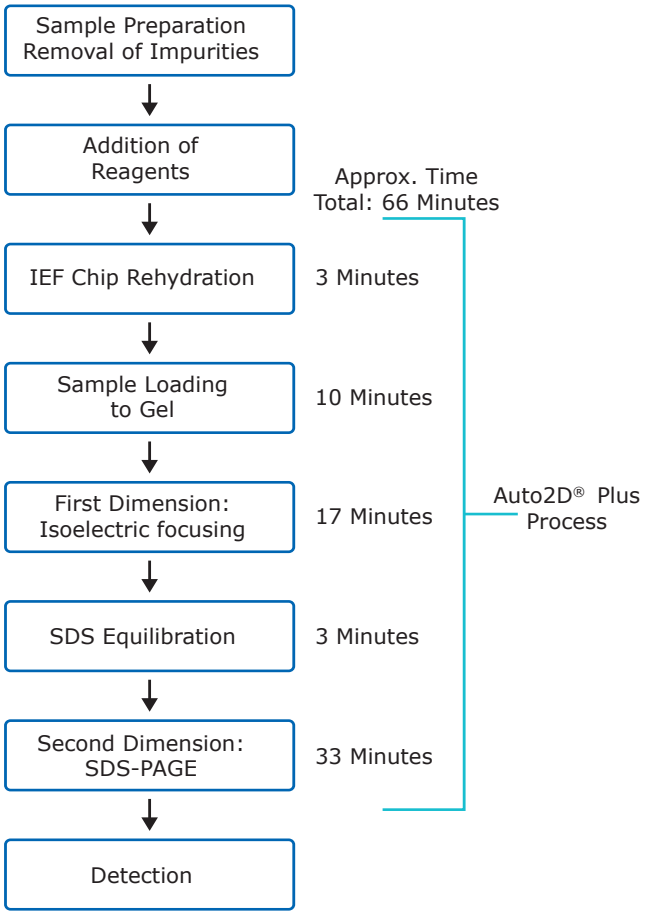
Auto2D® Plus Consumables

The Solution Chip Plus (Cat. No. BM-1SP) and the Electrode Chip Plus (Cat. No. BM-1EP) are specifically tailored to work with the Auto2D® Plus Electrophoresis process.

The PAGE Chips, IEF Chips and Reagent kits are compatible with either Plus mode or original mode.

Workflow

The Auto2D® Plus Electrophoresis Protocol produces high fidelity results in approximately 66–138 minutes depending on the recipe.



Preparation

Reagents

The Auto2D® Tris-Glycine Reagent Kit (Cat. No. BM-1RYSJ1) or Auto2D® Tris-Tricine Reagent Kit (Cat. No. BM-1RYTJ1) is needed for these steps.

Before first kit use, the Rehydration Solution and DTT Solution must be reconstituted as following:

Rehydration Solution

1. Add 6 mL of distilled water to the Rehydration Solution bottle and dissolve the powder completely. When completely dissolved, the total volume of Rehydration Solution will be 10 mL.
2. Aliquot Rehydration Solution into the supplied dispensing tubes, adding 189 µL* to each tube and store at -20 °C until use.

DTT Solution (1 M)

1. Add 3 mL of distilled water to the DTT bottle and dissolve the powder completely. When completely dissolved, the total volume of DTT Solution will be 3.5 mL.
2. Aliquot DTT Solution into the supplied dispensing tubes, 60 µL* each, and store at -20 °C until use.

* The amount of Rehydration Solution and DTT Solution required will change if Desalting recipes are used. In this case, adjust the aliquot volume to match the required Solution volume for one Auto2D® Electrophoresis run.

Anode Buffer

It is recommended to add the SDS to the Anode Buffer bottle so that its final concentration is 0.05%.

Auto2D® Chips and Solution Equilibration

Allow the following products stored at low temperatures to equilibrate to room temperature (20–25 °C for approximately 10 minutes before use).

- IEF Chip
- PAGE Chip
- Rehydration Solution
- DTT Solution
- Equilibriation Buffer
- Cathode Buffer
- Anode Buffer
- Ampholyte

Working Solutions

The Working Rehydration Solution and Working Equilibration Buffer must be prepared fresh for each use.

Prepare the Working Rehydration Solution for sample preparation and IEF Chip rehydration.

Reagents	Volume	Final Concentration
Rehydration Solution	189 µL	
DTT Solution (1M)	10 µL	50 mM
Ampholyte*	1–2 µL	0.5–1% v/v
Total	**200 µL**	

* Select ampholyte according to the range of IEF Chip used. For ampholytes at 40% stock concentration, add 1 µL. For ampholytes at 100X, add 2 µL.

Prepare the Working Equilibration Buffer for equilibration of focused proteins before SDS-PAGE.

Reagents	Volume	Final Concentration
Equilibration buffer premix	760 µL	
DTT Solution (1M)	40 µL	50 mM
Total	**800 µL**	

Sample Preparation

Quantify Proteins

Between 0.1–100 µg of protein can be loaded for 2D electrophoresis. The optimum amount of protein will depend on detection method and sample complexity. The following protein amounts should be used as a starting point, users may need to optimize the loading amount for their particular sample.

- Coomassie Brilliant Blue staining: 50 µg
- Fluorescent staining: 10 µg
- Silver staining: 5 µg
- Fluorescent pre-labeling: 3 µg

Use the amount of protein loaded into Solution Chip to determine whether to use S, M, or L recipe type for protein separation.

Dissolve the protein sample in the Working Rehydration Solution, prepared as described above. Sample may be diluted 2-fold or more with Working Rehydration Solution to reach desired protein concentration and decrease salt concentration.

A high salt concentration can affect protein separation during isoelectric focusing and cause the current to exceed 100 µA. Samples with a high salt concentration should be desalted by either:

TCA/acetone precipitation and resuspension of proteins in working rehydration solution

OR

Buffer exchange using a spin column

OR

Desalting protocol using Auto2D® Device

Sample Solution (13–15 µL)

Protein Sample	1–7 µL
Working Rehydration Solution (prepared in above step)	X µL
Total	**13–15 µL**

This solution should be prepared the day of use.

NOTE: During protein resuspension, avoid heating proteins over 37 °C.

Device Setup

Turn on Auto2D® Device

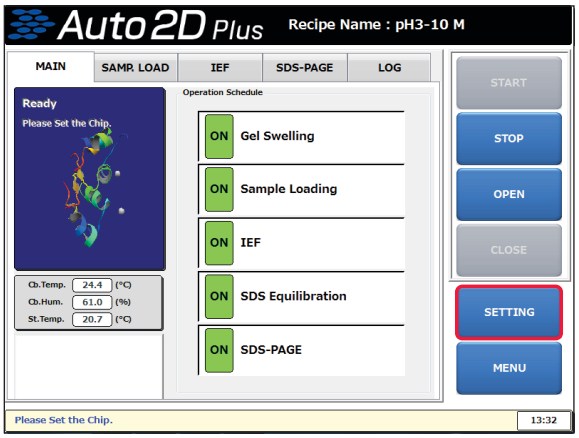
Turn on the power switch at the back of the device (see *Auto2D® Device Features on page 4)*.
The application should launch automatically.

From the main screen, touch [Auto2D Plus] to launch Auto2D® Plus mode.

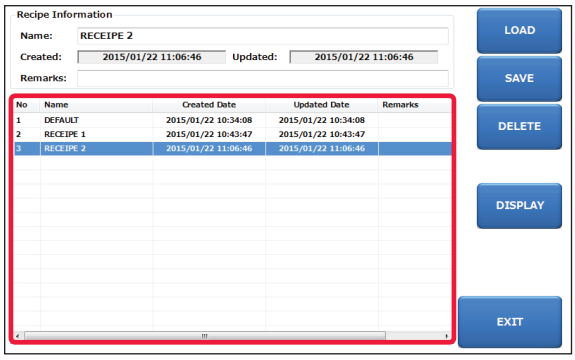
Set the Conditions

See *Auto2D® Plus Recipes beginning on page 22,* for recommended recipes.

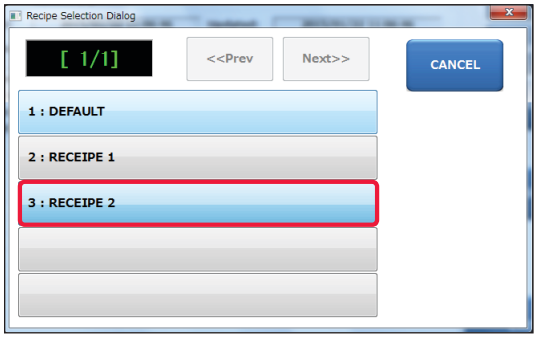
1. Touch the [SETTING] button on the Main Menu. Settings screen will appear.



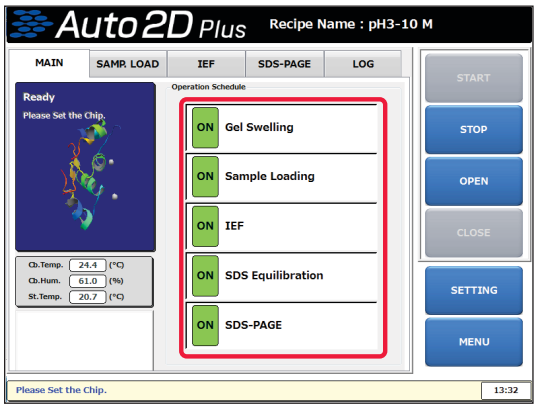
2. Load a Recipe
   A. Touch the [RECIPE] button.
   B. Touch the red framed area on the Recipe Information screen. The Recipe Select Dialog will appear.



3. Select the desired recipe. Auto2D® Plus Mode has recipes pre-installed. For a list of recipes or to create a new recipe, see *page 20.*



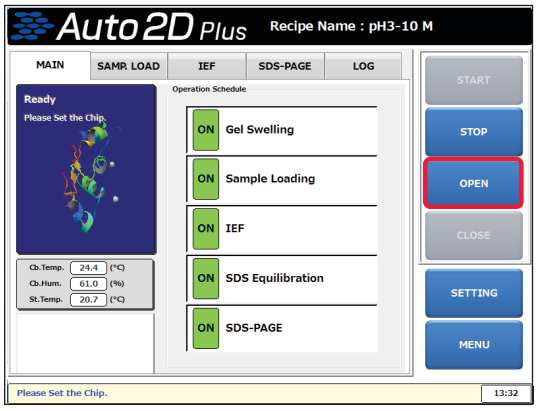
4. Touch the [LOAD] button to load selected recipe for use. Next touch [OK] > [EXIT] to return to the Auto2D® Plus mode operation screen. The selected recipe name should now appear at the top of the display screen.
5. Set the Operation Schedule
   A. Touch and turn ON/OFF each process on Main screen.



NOTE: Normal 2D electrophoresis uses all the processes.

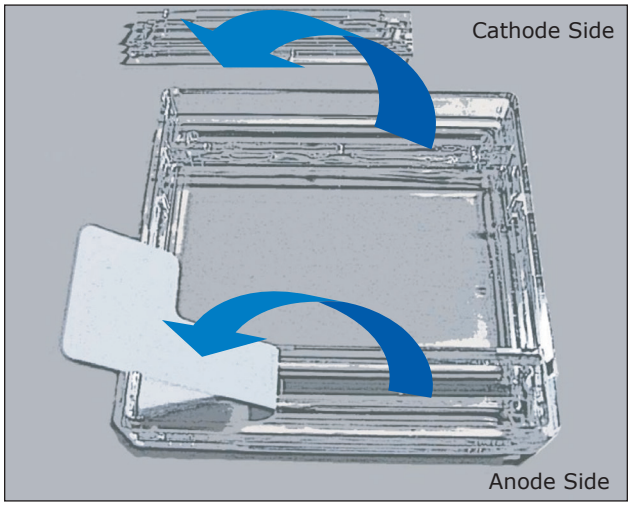
Open the Tray

On the Auto2D® Device touch screen, Touch [OPEN] > [OK].



Installing Chips and Solutions

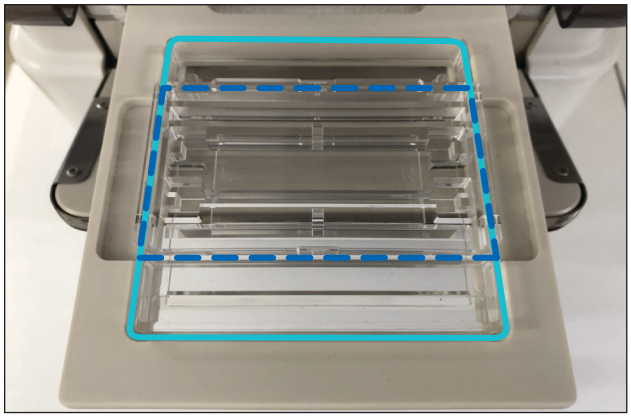
Install the PAGE Chip



1. Remove the white tape on the anode side of the PAGE Chip.

   NOTE: If PAGE Chip has not equilibriated to room temperature, the white tape may tear during removal.

2. Carefully remove the plastic cover on the cathode side of the Chip.
3. Gently rinse anode and cathode buffer wells with distilled water. Using a paper cleaning wipe, carefully wipe any liquid from the top of the Chip making sure not to damage the connecting gel located on the cathode side of the Chip.

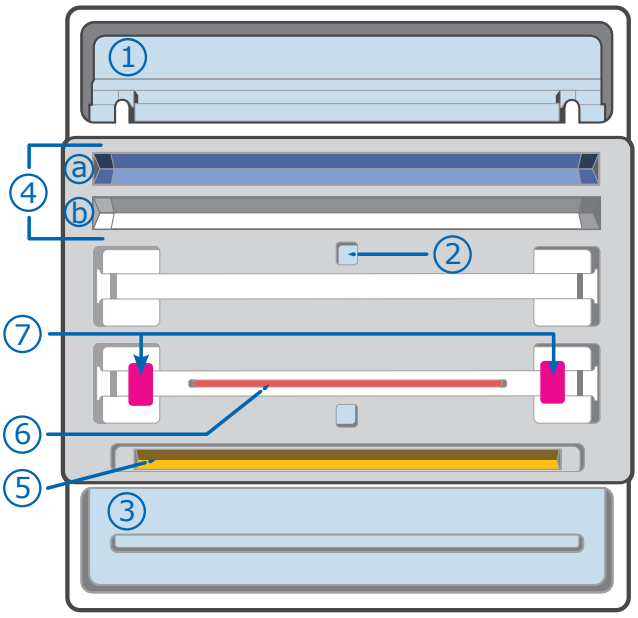


Once tray is open, place the PAGE Chip (light blue line) with the anode side in front. Place the Solution Chip Plus (dark blue lines) on top of the PAGE Chip with the cut-off corners in the front.

Apply the Solutions

A diagram indicating reagent loading locations and volumes will be displayed on the Auto2D® Device display when the door is opened. Apply reagent and sample solutions in the grooves in the order listed below.

NOTE: Apply each reagent and solution uniformly in their respective groove of the Solution Chip Plus.



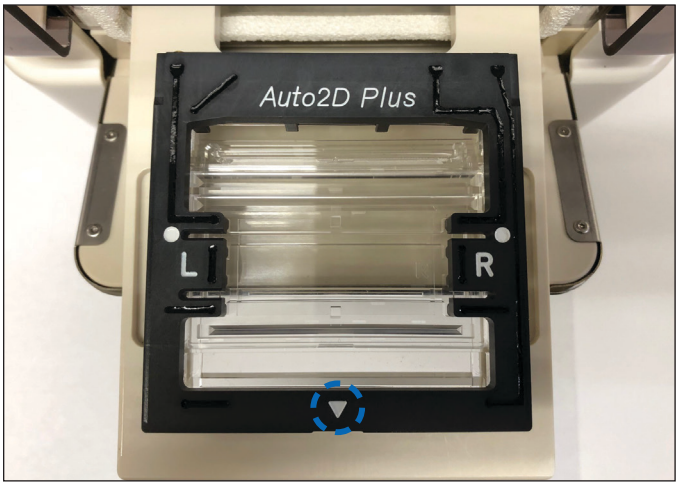
1. Cathode Buffer, 4500 µL
2. Distilled Water, 4500 µL
3. Anode Buffer, 4000 µL
4. Working Equilibration Buffer:
   a. 700 µL
   b. 700 µL (optional)
5. Working Rehydration Solution, 100 µL
6. Sample, 13–15 µL
7. Filter Paper and Water* 5 µL, each (Optional)

   * Wetted filter paper can be used as a wick to trap salt. Using filter paper is recommended to enhance separation of protein samples containing a high salt concentration when using Auto2D® Plus programs and is required when using Auto2D® Desalting program. Filter paper should be 0.2–0.3 mm in thickness and cut to a size of 8 mm x 4 mm.

Install the Electrode Chip Plus

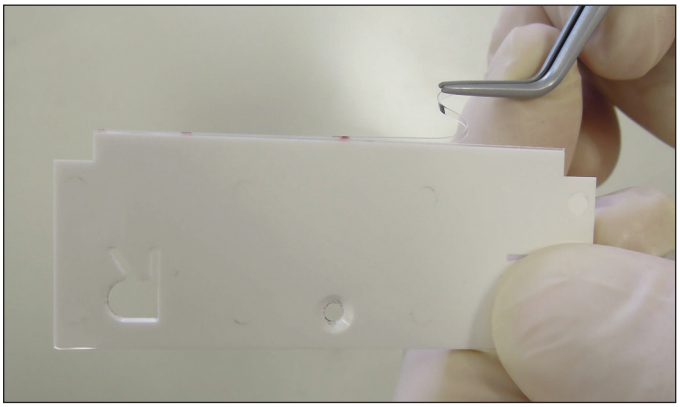
Before starting, inspect the wire of the Electrode Chip Plus for possible damage. Do not use if damaged. Electrode Chip Plus should be clean and dry.

Place over Solution Chip Plus.

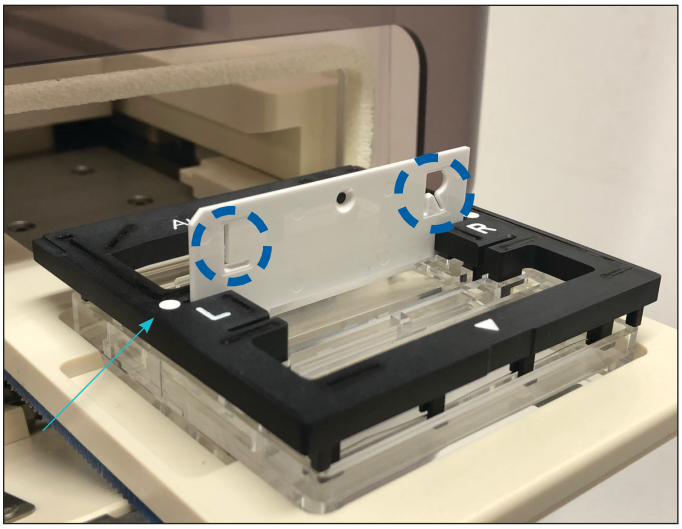


Position the ▼ towards the front.

Install the IEF Chip



Remove the IEF Chip protective film.



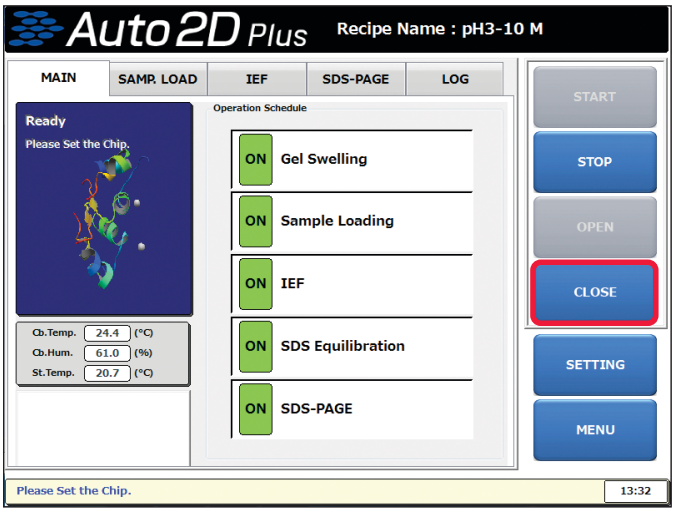
Insert the IEF Chip in the slot indicated by the white dot on the Electrode Chip Plus. The "L" should be on the left and "R" should be on the right when facing the device.

Before the Starting Eletrophoresis step, verify the following:

- Electrode Chip Plus is not wet except for anode and cathode chamber.
- The clear cover on the cathode side of the PAGE Chip is removed.
- Solution has been added where required.

Close the Tray

Touch [CLOSE] > [OK] on Main Menu. The Chip tray will be loaded into Auto2D® Device automatically.

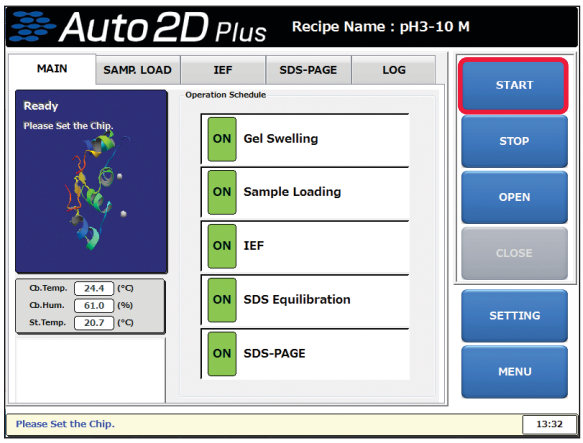


Steps of Operation

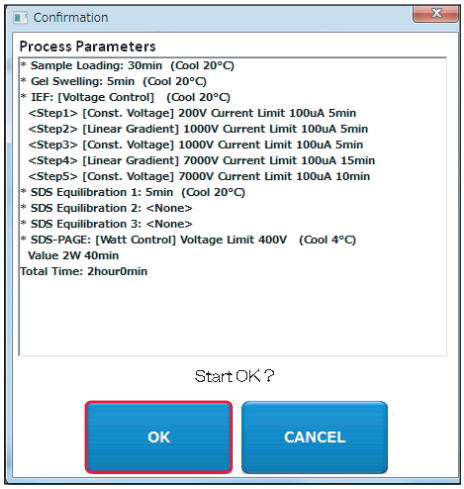
Start Electrophoresis

Touch [START] > [OK].

1. Touch [START] button on Main Menu.



2. Process Parameters Confirmation window will be displayed. Check each listed item carefully and touch [OK] button if all are OK. Electrophoresis will start automatically.

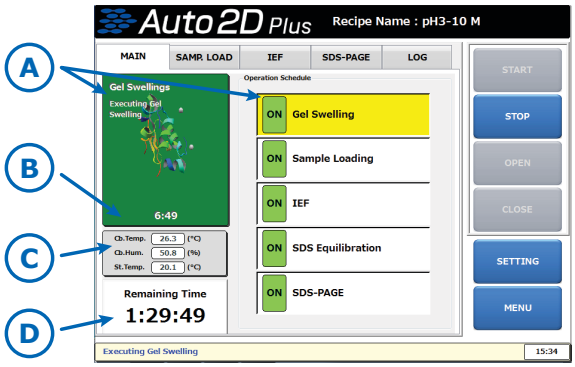


NOTE: All data of voltage, current, temperature, and humidity can be monitored and saved in the SSD as a CSV file.

Screens During Electrophoresis

You can touch each tab to display a real time graph of voltage and current values for each process.

MAIN Tab



A. Current Process

B. Current Process Remaining Time

C. Electrophoresis Chamber Data

D. Electrophoresis Remaining Time

Electrophoresis Chamber Data

Cb. Temp.: Chamber Internal Temperature

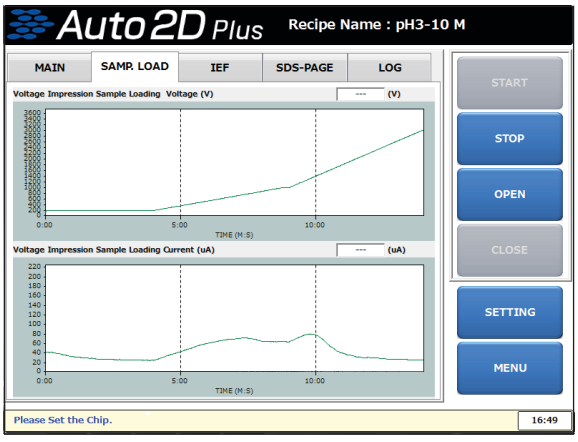
Cb. Hum.: Chamber Internal Humidity

St. Temp.: Stage Surface Temperature

CAUTION: The Humidity display turns red when the humidity drops to 40% or lower. Electrophoresis results are negatively impacted when the humidity is low and may cause evaporation of the reagents.

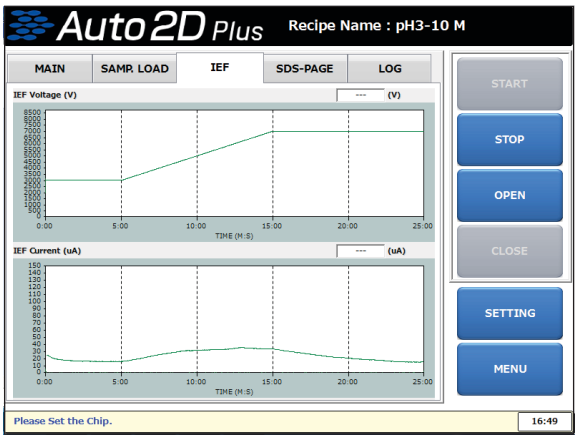
SAMPLE LOADING Tab

Graphs of Time vs. Voltage and Time vs. Current allow you to monitor the sample loading process.



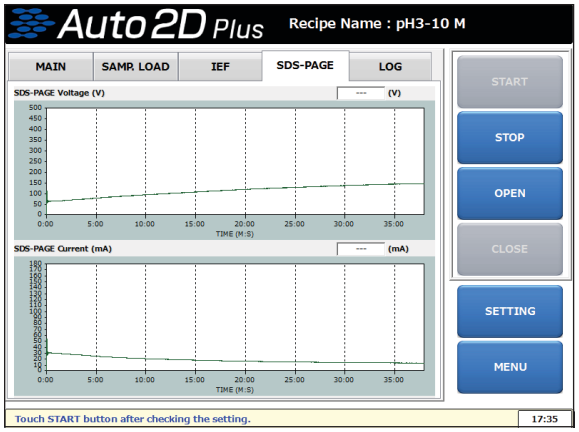
IEF Tab

Graphs of Time vs. Voltage and Time vs. Current allow you to monitor the IEF process.



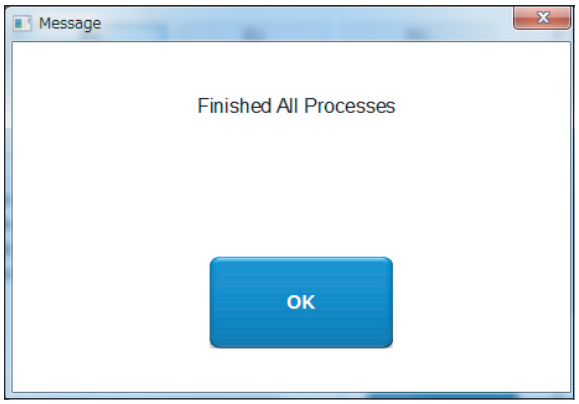
SDS-PAGE Tab

Graphs of Time vs. Voltage and Time vs. Current allow you to monitor the SDS-PAGE process.



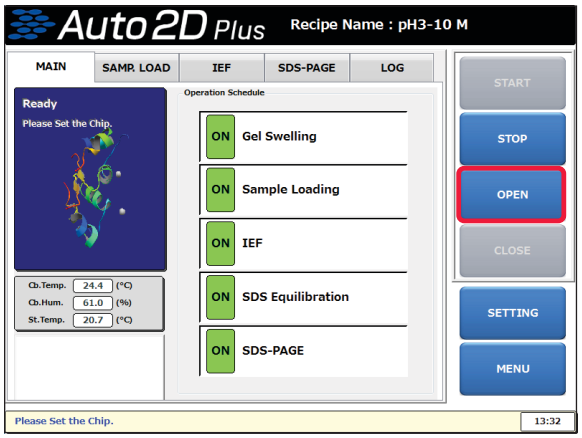
Finish Electrophoresis

The Auto2D® Device screen will confirm the electrophoresis program is finished.



Remove Chip Assembly

1. Touch [OPEN] button on the Main Menu.



2. Remove the Electrode Chip Plus.
3. Remove the Solution Chip Plus being careful not to spill the reagents and dispose any remaining solutions according to your local regulations.

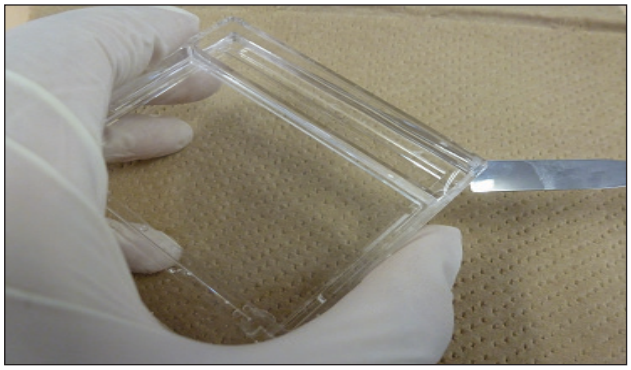
Remove Gel from PAGE Chip

When fluorescence imaging system (either scanner type or camera type) is available and sample is labeled with a fluorescence dye prior to electrophoresis, the PAGE Chip can be scanned directly without removing the gel from the Chip.

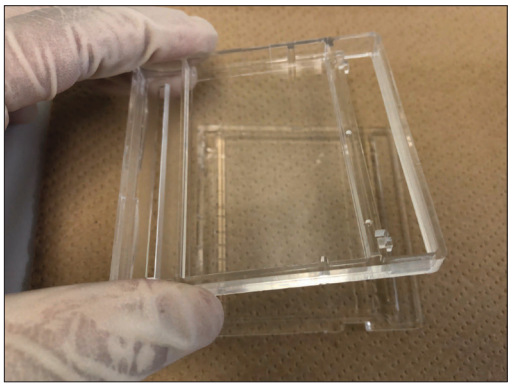
For other detection methods (staining methods or downstream applications), open the Chip following the instructions below.

1. Disassemble PAGE Chip.

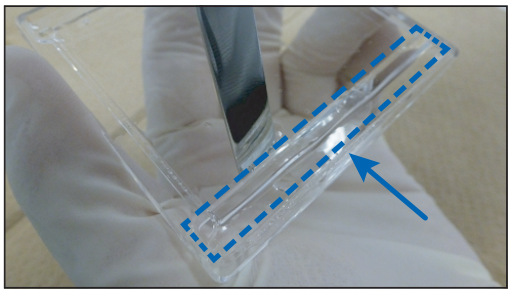
   Insert the tip of a metal spatula between the top cover and Chip body at the 4 corners of the PAGE Chip and slightly twist to separate. Noise will be heard at the time of separation.



Slowly separate the top cover from the Chip body while taking care not to tear or damage the gel.



2. Remove excess gel.

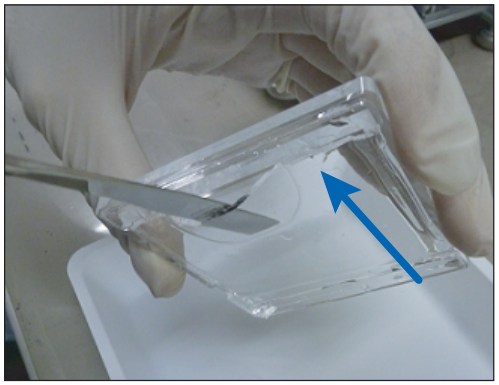


Excess gel lip at Anode side (Block shaped gel)

Before imaging the gel, remove the excess off the lip on the anode side, being careful not to remove any stained section of the gel.

NOTE: Remove the connecting gel at Cathode side when you transfer the proteins to membrane, because the connecting gel sticks to membrane.

3. Remove Remaining Gel from PAGE Chip



Insert a metal spatula under the gel at anode side and remove the whole gel from the PAGE Chip body.

Use spatula to cut a small piece from one corner of the gel. Record which corner and use this to orient the gel during staining or Western blotting.

TIP: Always remove the gel starting from the anode side to avoid damaging the separation area.

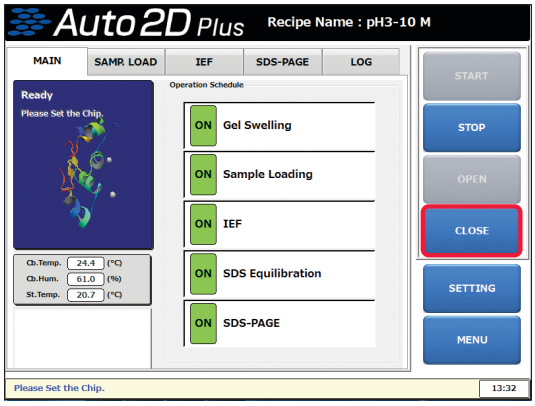
4. Proceed to gel staining or other analysis process.

Cleaning Electrode Chip

Clean the Electrode Chip Plus with distilled water immediately after use. Allow it to air dry.

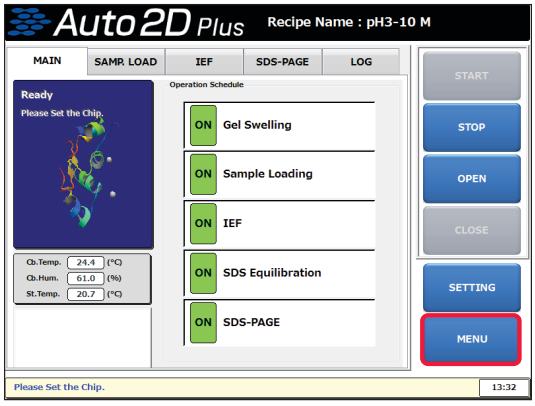
Turn off the Auto2D® Device

1. Touch the [CLOSE] button on the Main Menu to close the Chip tray.



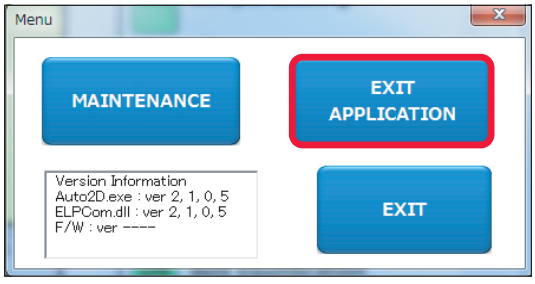
NOTE: If there is condensation inside device, leave tray opened until the inside dries. When the machine is dry turn the power off.

2. Touch the [MENU] button on the Main Menu. Menu window will pop up.



3. Touch [EXIT APPLICATION] > [OK] on the Menu window. This will open the Starting Screen. Touch [System/OS Shutdown] > [OK].

The screen will turn off.



4. Turn off the Power Switch on the back of the Auto2D® Device (see *page 4*).

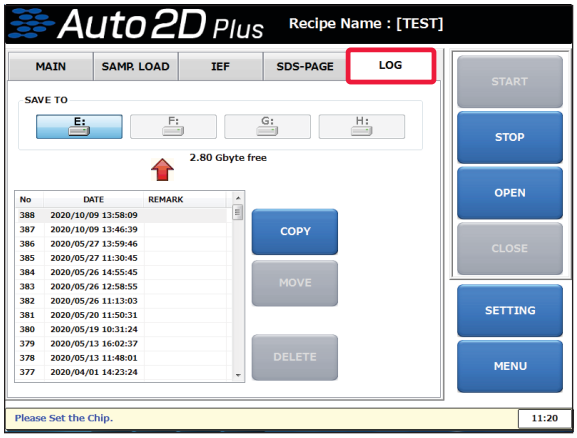
   A fan within Auto2D® Device continues to run until the power switch is turned off. Be sure to turn off the Power Switch.

   CAUTION: In rare cases, the system will not boot up properly if not turned off correctly. Please turn off according to the instructions to avoid persistent issues or damage to the Auto2D® Device. In the case the main power is on but the system does not start up, there is a reset switch on the lower right of the touch panel. For help contact Technical Service at SigmaAldrich.com/techservice.

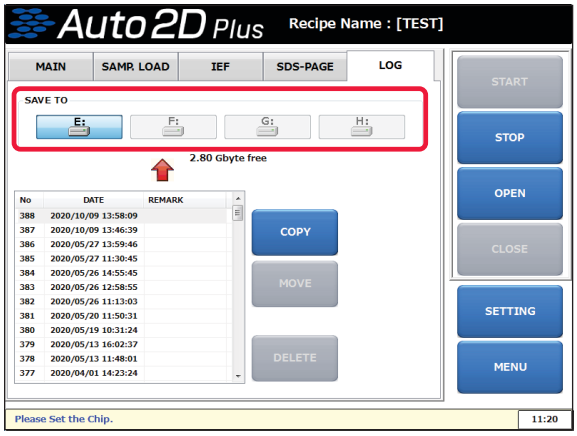
Saving Data

To Copy the Log

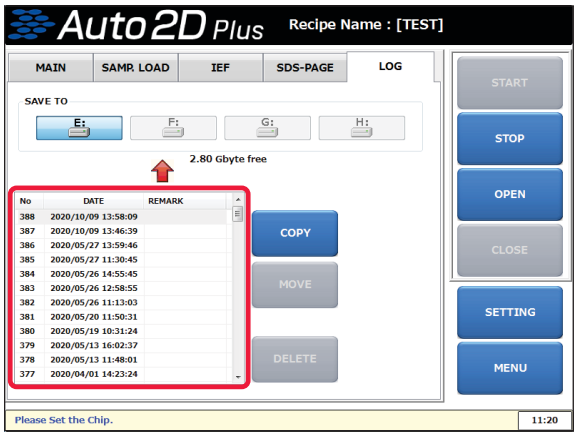
1. Connect a USB flash drive to the USB port on the right side of the Control Panel.
2. Touch the [LOG] tab on the Main Menu.



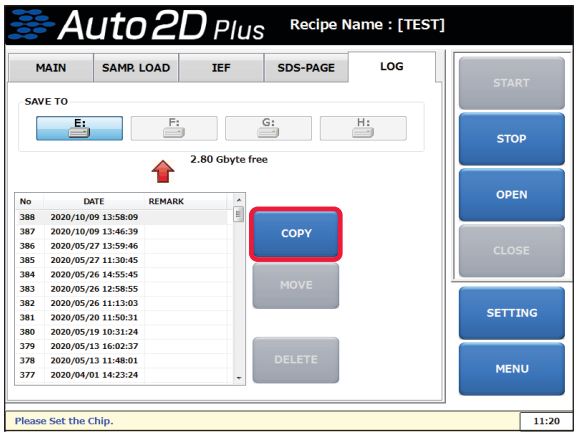
Select the drive to SAVE TO.



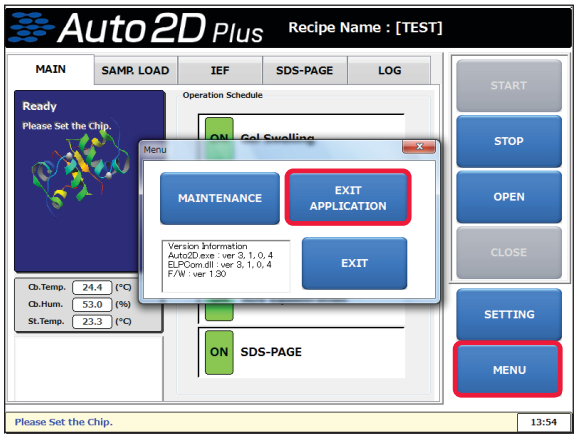
3. Touch and select a file to copy from the file list.



4. Touch the [COPY] button.
   Confirmation window will pop up.
   "Copied successfully." message will be displayed.



5. Touch the [MENU] button and then [EXIT APPLICATION] button. Touch the [System/OS shutdown] button. Turn off the Auto2D® Device using the power switch on the back of the device.



6. Remove the USB flash drive from the Auto2D® device.

   CAUTION: To avoid lost or damaged data, Auto2D® must be completely powered off before removing USB flash drive.

Desalting Process

Reagent Preparation

For instructions on the initial preparation of the Rehydration Solution and 1M DTT solution, see *page 10*.

Rehydration Solution 965 µL/run Composition: 8M Urea, 2M Thiourea, 4% CHAPS

Filter Paper Preparation

Filter paper (optional), 2 pieces/run, size:
Length: 8 mm
Width: 4 mm
Thickness: 0.2–0.3 mm

Working Reagent Preparation

Prepare **Working Rehydration Solution** as shown below.

Rehydration Solution	965 µL
1M DTT Solution	30 µL (30 mM)*
Ampholyte**	Volume: 5–10 µL.
Total Amount	**1 mL**

Application volume is 600 µL.

* The final concentration is different from standard recipes.

** Select ampholyte according to the range of IEF Chip to use. For ampholytes at 40% stock concentration, add 5 µL. For ampholytes at 100X, add 10 µL.

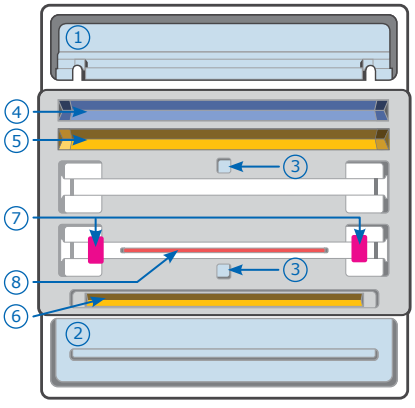
Prepare **Working Equilibration Buffer** as shown below.

Equilibration buffer premix	760 µL
DTT Solution (1M)	40 µL (50 mM)
Total Amount	**800 µL**

Load a Recipe

Desalting Recipes on page 29 lists a range of IEF Chip Recipes to use.

Desalting Solution Application



1. Cathode Buffer 4500 uL
2. Anode Buffer 4000 µL
3. Cooling (DI) Water 4500 µL
4. Equilibration Buffer 700 µL
5. Working Rehydration Solution 500 µL
6. Working Rehydration Solution 100 µL
7. Filter Paper + DI water 5 µL each
8. Sample 13–15 µL

Note: The Auto2D® device screen will only show the standard recipe solution positions. For Desalting, solutions must be applied as shown above.

Auto-Staining Process

Reagent Preparation

In addition to the normal upfront preparation reagents described earlier, prepare the following reagents:

- Dilution buffer
  Composition: 50mM Tris HCl pH 8.8, 1% SDS
- Labeling Dye: Dissolve to 1 µg/µL with DMSO.
  Suggested dyes are NHS ester dyes.

Prepare the **Labeling Solution** as shown below.

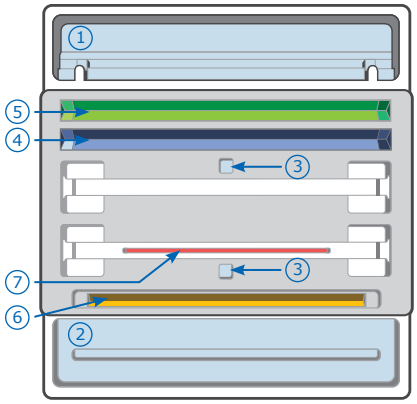
Dilution Buffer	495 µL
Labeling Dye	5 µL (10 ng/µL)
Total Amount	**500 µL**

Application volume is 500 µL.

Load a Recipe

Choose the range of IEF Chips to use from the recipes on *page 31*.

Auto-Staining Solution Application



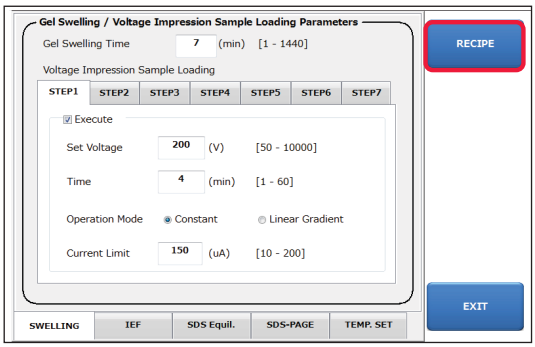
1. Cathode Buffer 4500 µL
2. Anode Buffer 4000 µL
3. Cooling (DI) water 4500 µL
4. Working Equilibration Buffer 700 µL
5. Labeling Solution 500 µL
6. Working Rehydration Solution 100 µL
7. Sample 13–15 µL

Note: The Auto2D® device screen will only show the standard recipe solution positions. For Auto-Staining, solutions must be applied as shown above.

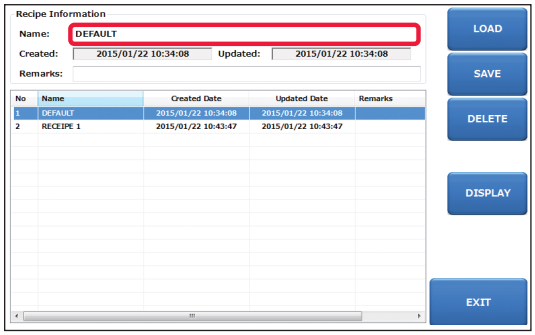
Make a New Recipe

This process is used for both Plus and Original modes.

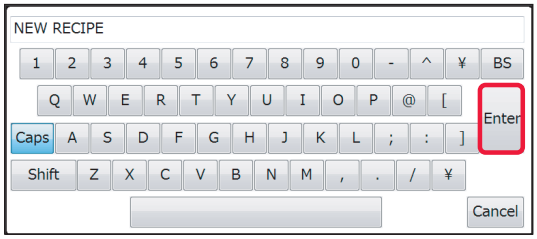
1. Touch the [RECIPE] button. When using the preset recipes, select "Load a Recipe"



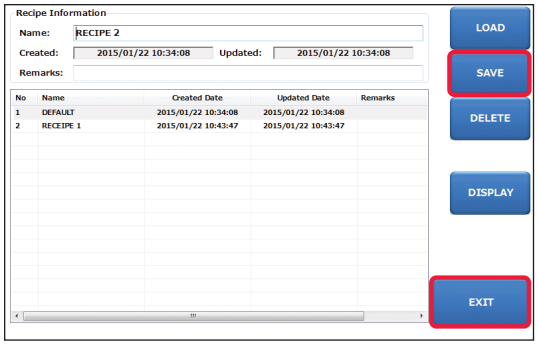
2. Touch the Name input box area.



3. Enter the Recipe Name with the key board and touch the [Enter] key.



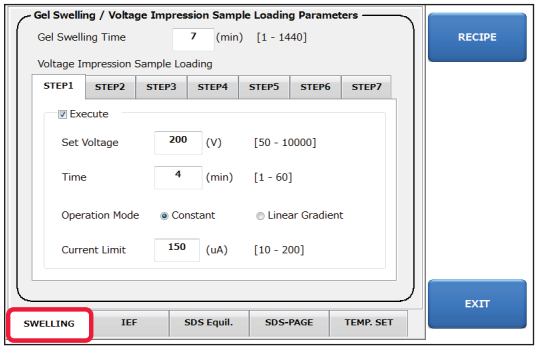
4. Touch the [SAVE] button.



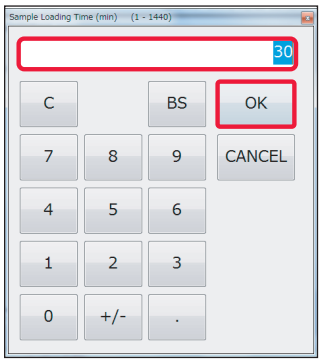
5. Touch the [EXIT] button.
   Setting screen will be displayed.

Set the Rehydration and Sample Loading Parameters

6. Touch the [SWELLING] tab.



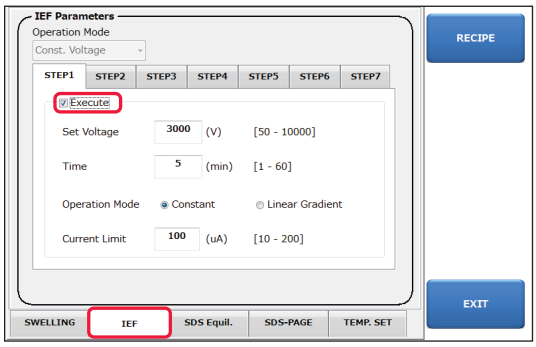
7. Set the Rehydration Time. > Touch the data input box. Keyboard will be displayed. Enter the value with the keyboard and touch [OK] key. Set the Set Voltage, Time, Operation Mode and Current Limit of each [STEP1] to [STEP7] tab.



Set the IEF Parameters

8. Touch the [IEF] tab.
9. Set the Set Voltage, Time, Operation Mode and Current Limit of each [STEP1] to [STEP7] tab.

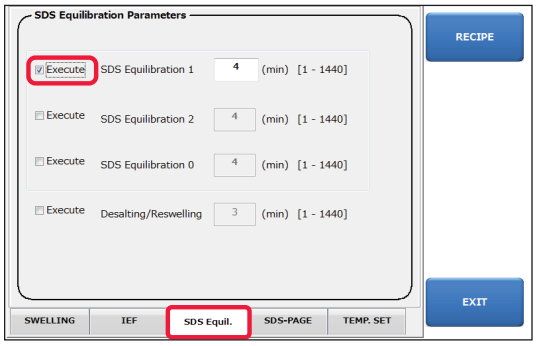
   NOTE: When number of STEPs needs to be reduced, uncheck the "Execute" check box above the Set Voltage of the respective [STEP] tab.



Set the SDS Equilibration Parameters

10. Touch the [SDS Equil.] tab.

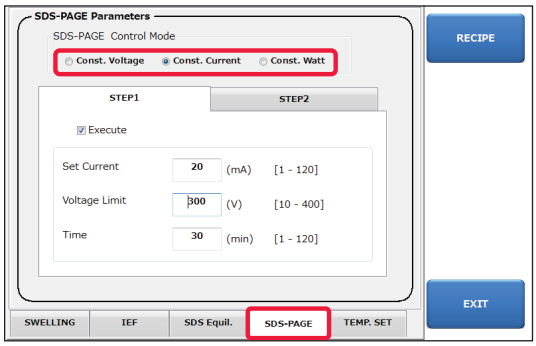
    Check or uncheck "Execute" check box and set the time for each SDS Equilibration 0 to 2 and Desalting/Reswelling.



NOTE: SDS Equilibration 0 is for pre-equilibration of Auto-Staining in anode chamber. Desalting/Reswelling is equilibration in the equilibration groove 2 before IEF process.

Set the PAGE Parameters

11. Touch the [SDS-PAGE] tab.
12. Select SDS PAGE Control Mode (Const. Voltage, Const. Current or Const. Watt).
13. Only when "Const. Current" is selected, set the value for each STEP 1 and 2.

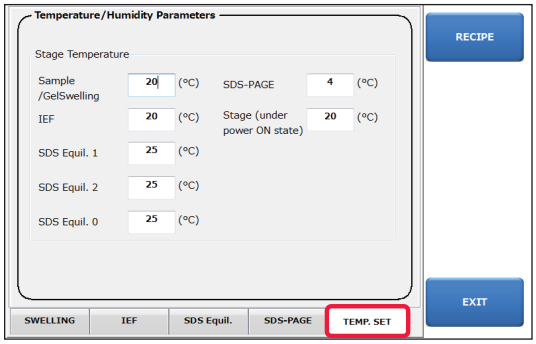


NOTE: Setting items varies depend on the Control Mode selected.

- Voltage control: Constant Voltage, Current limit and Time
- Current control: Constant Current, Voltage limit and Time
- Power control: Constant Watt, Voltage limit and Time

Set Temperature/Humidity Parameters

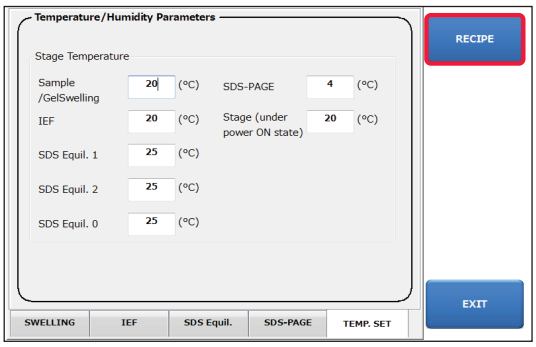
14. Touch the [TEMP. SET] tab.



15. Set the temperature of each process.

Save the setting

16. Touch the [RECIPE] button on the right.



17. Check the Recipe Name to save and touch the [SAVE] button.

    NOTE: A new recipe can be created by saving the recipe with a different name. If saved without changing the name, the changes made are overwritten to the recipe read just before making the changes.

18. Touch the [EXIT] button two times to go to the Main screen.

Auto2D® Plus Recipes

For standard 2D protein separation.

1. **pH (#–#) M:** Standard recipes, about 90 minutes.
2. **pH (#–#) S:** For trace samples of 10 µg or less, about 60 minutes.
3. **pH (#–#) L:** For when standard program does not provide enough focus, about 120 minutes.

pH 3–10 M

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 5 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 3–10 S

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 3–10 L

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 3–10 NL M

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 3–10 NL S

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 3–10 NL L

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 7000 V, 15 minutes, Linear Gradient
3. 7000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–7 M

Rehydration Time: 4 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–7 S

Rehydration Time: 3 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–7 L

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 7000 V, 15 minutes, Linear Gradient
3. 7000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 6–10 M

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 8000 V, 10 minutes, Linear Gradient
3. 8000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 6–10 S

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 5 minutes, Constant Voltage
2. 8000 V, 4 minutes, Linear Gradient
3. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 6–10 L

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 7000 V, 15 minutes, Linear Gradient
3. 7000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–5.5 M

Rehydration Time: 4 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 15 minutes, Linear Gradient
3. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–5.5 S

Rehydration Time: 3 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 5000 V, 4 minutes, Linear Gradient
3. 5000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 4–5.5 L

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 5000 V, 15 minutes, Linear Gradient
3. 5000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 5–6.5 M

Rehydration Time: 4 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 5–6.5 S

Rehydration Time: 3 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 5 minutes, Constant Voltage
2. 7000 V, 5 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 5–6.5 L

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 7000 V, 15 minutes, Linear Gradient
3. 7000 V, 25 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 7–10 M

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 4 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 10 minutes, Constant Voltage
2. 7000 V, 10 minutes, Linear Gradient
3. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 4 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 7–10 S

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 5 minutes, Constant Voltage
2. 8000 V, 4 minutes, Linear Gradient
3. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

pH 7–10 L

Rehydration Time: 15 minutes

Sample Loading
Current Limit: 150 µA

1. 100 V, 3 minutes, Constant Voltage
2. 200 V, 7 minutes, Linear Gradient
3. 1000 V, 10 minutes, Linear Gradient
4. 3000 V, 10 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 15 minutes, Constant Voltage
2. 8000 V, 15 minutes, Linear Gradient
3. 8000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalting Recipes

Desalt pH 3–10

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 15 minutes, Constant Voltage
2. 200 V, 20 minutes, Linear Gradient
3. 1000 V, 5 minutes, Linear Gradient
4. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 7000 V, 10 minutes, Linear Gradient
5. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalt pH 3–10 NL

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 15 minutes, Constant Voltage
2. 200 V, 20 minutes, Linear Gradient
3. 1000 V, 5 minutes, Linear Gradient
4. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 7000 V, 10 minutes, Linear Gradient
5. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Can not use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalt pH 4–7

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 15 minutes, Constant Voltage
2. 200 V, 20 minutes, Linear Gradient
3. 1000 V, 5 minutes, Linear Gradient
4. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 10 minutes, Linear Gradient
3. 1000 V, 10 minutes, Constant Voltage
4. 7000 V, 10 minutes, Linear Gradient
5. 7000 V, 15 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalt pH 6–10

Rehydration Time: 10 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 5 minutes, Constant Voltage
2. 200 V, 10 minutes, Linear Gradient
3. 200 V, 15 minutes, Constant Voltage
4. 1000 V, 5 minutes, Linear Gradient
5. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient
4. 3000 V, 5 minutes, Constant Voltage
5. 8000 V, 10 minutes, Linear Gradient
6. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalt pH 4–5.5

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 15 minutes, Constant Voltage
2. 200 V, 20 minutes, Linear Gradient
3. 1000 V, 5 minutes, Linear Gradient
4. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 3000 V, 10 minutes, Linear Gradient
3. 3000 V, 10 minutes, Constant Voltage
4. 5000 V, 10 minutes, Linear Gradient
5. 5000 V, 15 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Desalt pH 5–6.5

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 15 minutes, Constant Voltage
2. 200 V, 20 minutes, Linear Gradient
3. 1000 V, 5 minutes, Linear Gradient
4. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 3000 V, 10 minutes, Linear Gradient
3. 3000 V, 10 minutes, Constant Voltage
4. 7000 V, 10 minutes, Linear Gradient
5. 7000 V, 20 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power

- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Staining Recipes

Desalt pH 7–10

Rehydration Time: 15 minutes

Sample Loading
Current Limit: 200 µA

1. 100 V, 5 minutes, Constant Voltage
2. 200 V, 10 minutes, Linear Gradient
3. 200 V, 15 minutes, Constant Voltage
4. 1000 V, 5 minutes, Linear Gradient
5. 1500 V, 5 minutes, Linear Gradient

Desalting
Desalting Time: 5 minutes

IEF
Current Limit: 100 µA

1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 3000 V, 5 minutes, Linear Gradient
4. 3000 V, 5 minutes, Constant Voltage
5. 8000 V, 10 minutes, Linear Gradient
6. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 3 minutes
- Equilibration Time 2: Cannot use
- Equilibration Time 0: Not used

PAGE
Mode: Constant Power
- Set Power: 2.0 W
- Voltage Limit: 400 V
- Electrophoresis Time: 38 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 3–10

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power
- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 3–10 NL

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power
- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 4–7

Rehydration Time: 3 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 6–10

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 8000 V, 4 minutes, Linear Gradient
3. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 4–5.5

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 5000 V, 4 minutes, Linear Gradient
3. 5000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 5–6.5

Rehydration Time: 5 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 7000 V, 4 minutes, Linear Gradient
3. 7000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto-Stain pH 7–10

Rehydration Time: 7 minutes

Sample Loading
Current Limit: 150 µA

1. 200 V, 2 minutes, Constant Voltage
2. 1000 V, 4 minutes, Linear Gradient
3. 3000 V, 4 minutes, Linear Gradient

IEF
Current Limit: 100 µA

1. 3000 V, 3 minutes, Constant Voltage
2. 8000 V, 4 minutes, Linear Gradient
3. 8000 V, 10 minutes, Constant Voltage

SDS Equilibrium

- Equilibration Time 1: 15 minutes
- Equilibration Time 2: 3 minutes
- Equilibration Time 0: 2 minutes

PAGE
Mode: Constant Power

- Set Power: 2.5 W
- Voltage Limit: 400 V
- Electrophoresis Time: 33 minutes

Temperature Setting

- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 25 °C
- Equilibration 0 Temperature: 25 °C

Auto2D® Mode (Original)

For our legacy customers, the Auto2D® mode (original) is still available. Auto2D® Plus mode is highly recommended unless you need to keep consistency with past data obtained by Auto2D® mode (original).

The Auto2D® Mode (original) fully automates the process of 2D electrophoresis, separating proteins first by isoelectric point and second by molecular weight. Complete protein separation is achieved in about two hours depending on the Recipe.

Auto2D® Plus Mode on page 9.

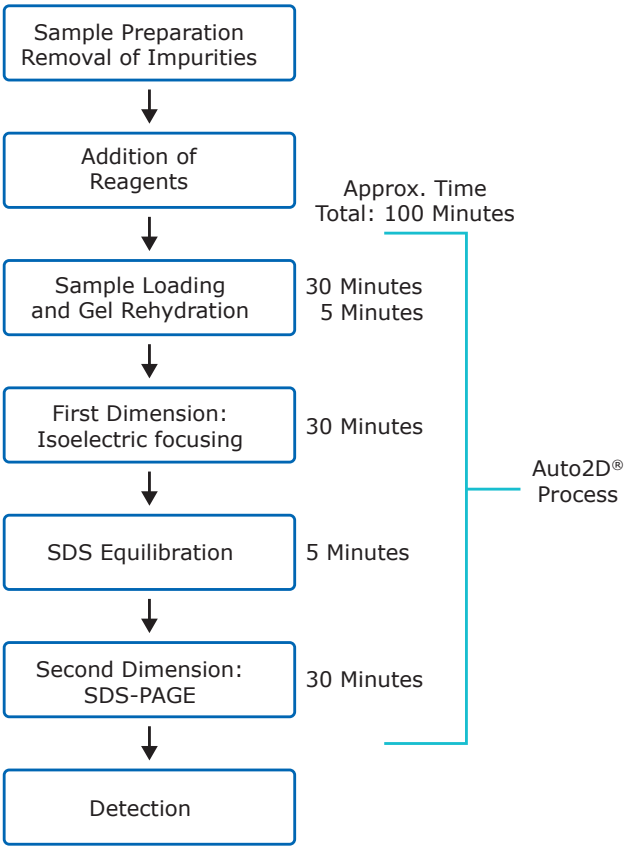
Auto2D® Mode (Original) Consumables

The Solution Chip (Cat. No. BM-1S) and the Electrode Chip (Cat. No. BM-1E) are specifically tailored to work with the Auto2D® (original mode) Electrophoresis process.

The PAGE Chips, IEF Chips and Reagent kits are compatible with either Plus mode or original mode.

Workflow

Auto2D® Device is more automated and less time consuming than conventional 2D Electrophoresis systems. High fidelity results are produced in approximately 100 minutes.



Preparation

Reagents

The Auto2D® Tris-Glycine Reagent Kit (Cat. No. BM-1RYSJ1) or Auto2D® Tris-Tricine Reagent Kit (Cat. No. BM-1RYTJ1) is needed for these steps.

Before first kit use, the Rehydration Solution and DTT Solution must be reconstituted as following:

Rehydration Solution

1. Add 6 mL of distilled water to the Rehydration Solution bottle and dissolve the powder completely. When completely dissolved, the total volume of Rehydration Solution will be 10 mL.
2. Aliquot Rehydration Solution into the supplied dispensing tubes, adding 189 µL to each tube and store at -20 °C until use.

DTT Solution (1 M)

1. Add 3 mL of distilled water to the DTT bottle and dissolve the powder completely. When completely dissolved, the total volume of DTT Solution will be 3.5 mL.
2. Aliquot DTT Solution into the supplied dispensing tubes, 60 µL each, and store at -20 °C until use.

Anode Buffer

It is recommended to add the SDS to the Anode Buffer bottle so that its final concentration is 0.05%.

Auto2D® Chips and Solution Equilibration

Allow the following products stored at low temperatures to equilibrate to room temperature (20–25 °C for approximately 10 minutes before use).

- IEF Chip
- PAGE Chip
- Rehydration Solution
- DTT Solution
- Equilibriation Buffer
- Cathode Buffer
- Anode Buffer
- Ampholyte

Working Solutions

Prepare the Working Rehydration Solution for rehydration of IEF Chip and extraction/dilution of protein sample:

Reagents	Volume	Final Concentration
Rehydration Solution	189 µL	
DTT Solution (1M)	10 µL	50 mM
Ampholyte*	1–2 µL	0.5–1% v/v
Total	**200 µL**	

* Select ampholyte according to the range of IEF Chip used. For ampholytes at 40% stock concentration, add 1 µL. For ampholytes at 100X, add 2 µL.

Prepare the Working Equilibration Buffer for equilibration of focused proteins before SDS-PAGE.

Reagents	Volume	Final Concentration
Equilibration buffer premix	760 µL	
DTT Solution (1M)	40 µL	50 mM
Total	**800 µL**	

Sample Preparation

Quantify Proteins

Between 0.1–25 µg of protein can be loaded for 2D electrophoresis. The optimum amount of protein will depend on detection method and sample complexity. The following protein amounts should be used as a starting point, users may need to optimize the loading amount for their particular sample.

- Coomassie Brilliant Blue staining: 25 µg
- Fluorescent staining: 10 µg
- Silver staining: 5 µg
- Fluorescent pre-labeling: 3 µg

Dissolve the protein sample in the Working Rehydration Solution, prepared as described above. Sample may be diluted 2-fold or more with Working Rehydration Solution to reach desired protein concentration and decrease salt concentration.

A high salt concentration can affect protein separation during isoelectric focusing and cause the current to exceed 100 µA. Samples with a high salt concentration should be desalted by either:

TCA/acetone precipitation and resuspension of proteins in working rehydration solution

OR

Buffer exchange using a spin column

OR

Desalting protocol using Auto2D® Device

Sample Solution (10–12 µL)

Protein Sample	1–6 µL
Working Rehydration Solution (prepared in above step)	X µL
Total	**10–12 µL**

This solution should be prepared the day of use.

NOTE: During protein resuspension, avoid heating proteins over 37 °C.

Device Setup

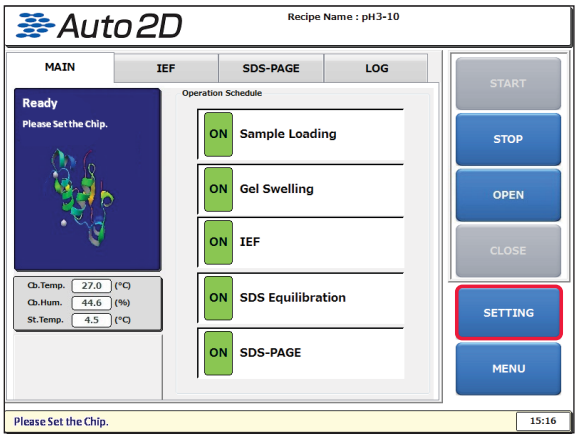
Turn on Auto2D® Device

Turn on the power switch at the back of the device. The application should launch automatically. From the Main screen, touch Auto2D® to launch Auto2D® original mode.

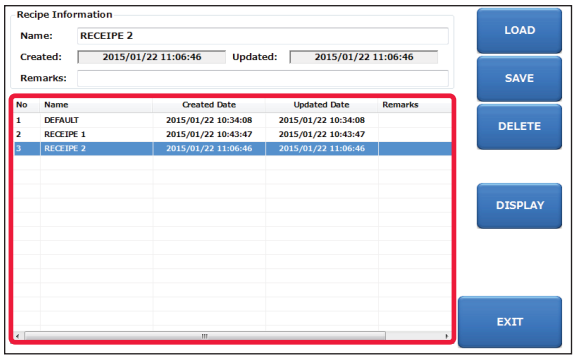
Set the Conditions

See *page 45* for recommended recipes.

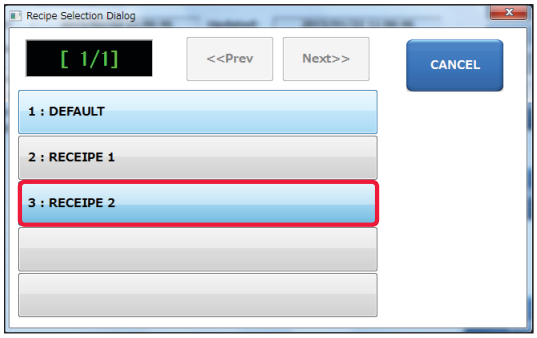
1. Touch the [SETTING] button on the Main Menu. Settings screen will appear.



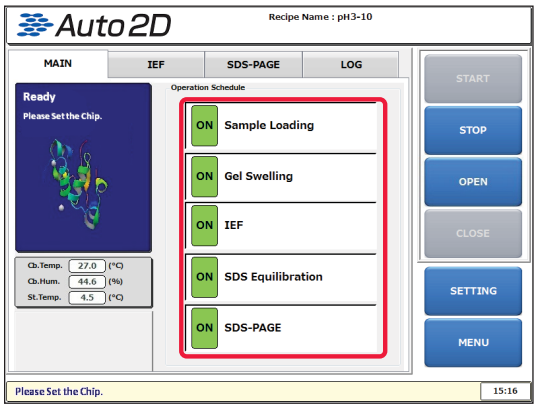
2. Load a Recipe
   A. Touch the [RECIPE] button.
   B. Touch the red framed area on the Recipe Information screen. The Recipe Select Dialog will appear.



3. Select a desired recipe. To create a new recipe see *Make a New Recipe beginning on page 20*.



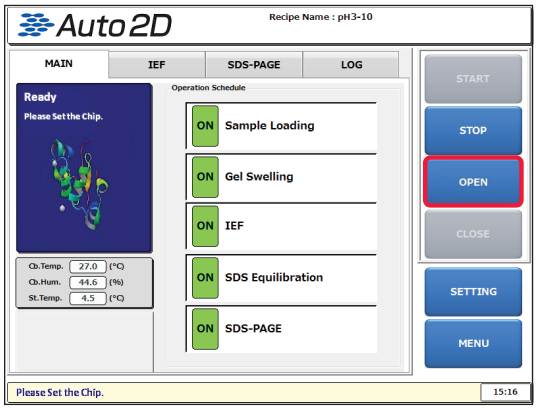
4. Touch the [LOAD] button to load selected recipe for use.
5. Set the Operation Schedule
   A. Touch and turn ON/OFF each process on the Main screen.



NOTE: Normal 2D electrophoresis uses all the processes.

Open the Tray

Touch the [OPEN] button on the Main Menu to open the tray.

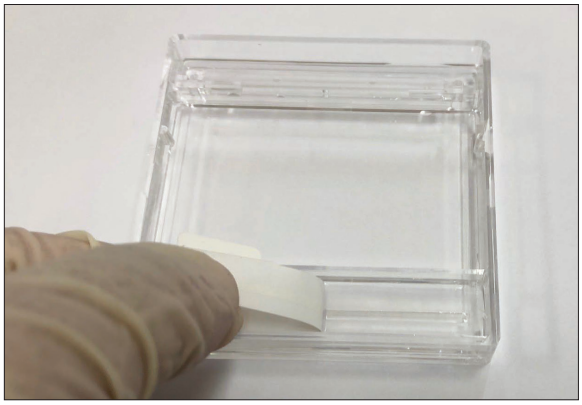


Installing Chips and Solutions

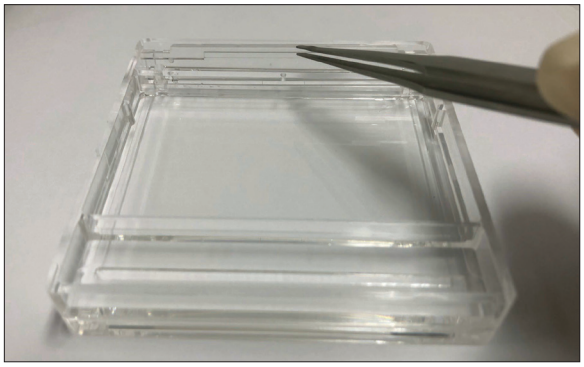
Install the PAGE Chip

1. Carefully remove a PAGE Chip from its pouch and gently wipe off any liquid around the Chip using a lint-free cleaning wipe.
2. Slowly remove the white tape at the anode side of the PAGE Chip.

   NOTE: If PAGE Chip has not equilibrated to room temperature, the white tape may tear during removal.

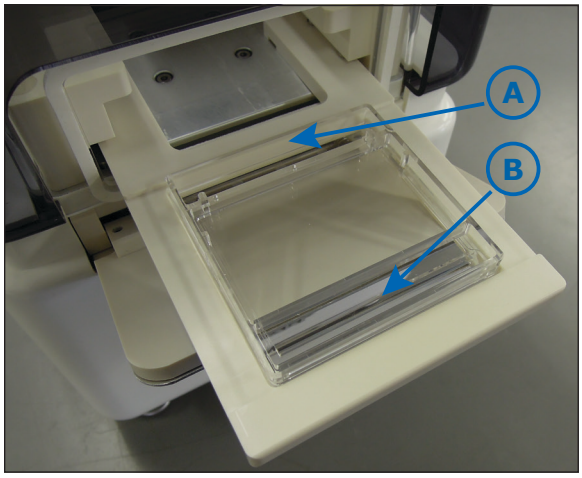


3. Remove the protective plastic cap at the cathode side of the PAGE Chip with tweezers or fingers, taking care not to damage the connecting gel protruding from the cassette.



4. Gently rinse the cathode side with distilled water 1 to 2 times to remove gel debris. Carefully wipe any liquid from the top, bottom, and sides of the Chip with a lint-free cleaning wipe, taking care not to damage the gel.
5. Place the PAGE Chip into the open Auto2D® device tray with the anode side in front.

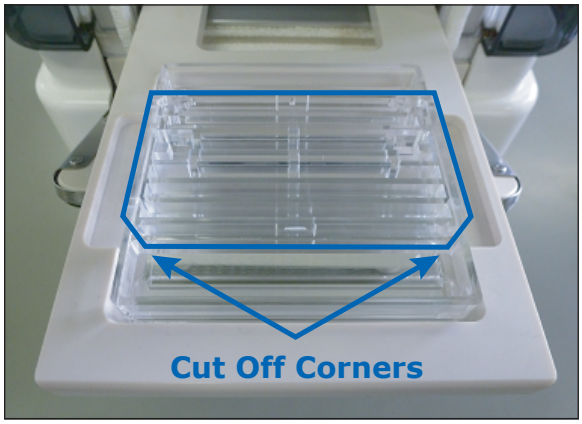
   Note: If the PAGE Chip is placed with the cathode side facing front, the Chip will not fit properly into tray. Correct orientation should be clear.



A. Cathode Side
B. Anode Side

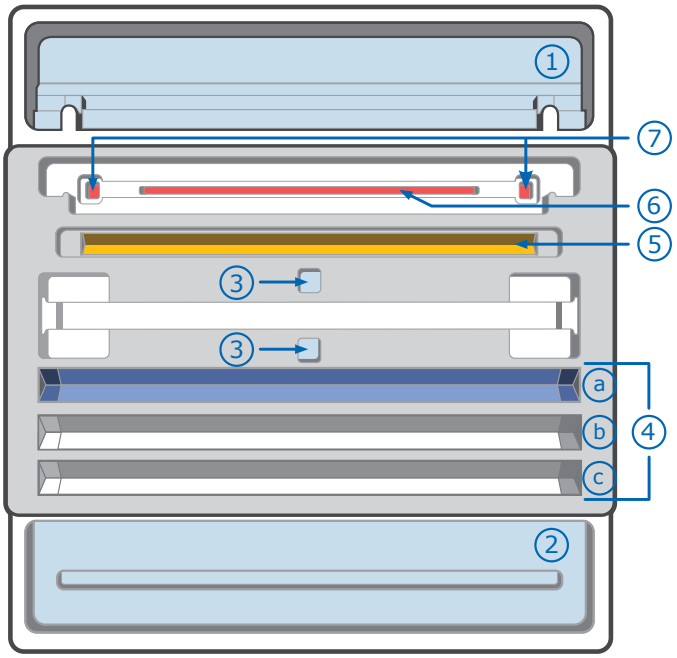
Install the Solution Chip

Remove the Solution Chip from its pouch and place it over the PAGE Chip such that its cut-off corners faces front.



Apply the Solutions

Apply solutions and buffers to Solution Chip.

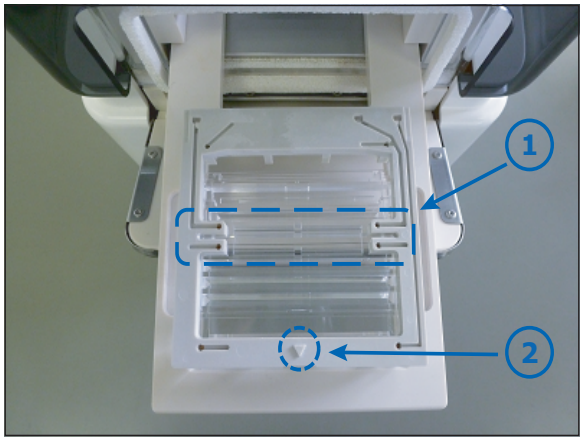


1. Cathode Buffer, 4500 µL
2. Anode Buffer, 4000 µL
3. Distilled Water, 4000 µL
4. Working Equilibration Buffer
   a. 700 µL
   b. 700 µL (optional)
   c. 700 µL (optional)
5. Working Rehydration Solution, 100 µL
6. Sample Solution, 10–12 µL
7. Molecular Weight Marker*, 0.5–0.7 µL

   * Cannot include SDS or Bromophenol Blue

Install the Electrode Chip (BM-1E)

Set the Electrode Chip onto the assembled gel and Solutions Chip so the delta shaped marking faces front.



1. IEF Electrode portion of Electrode Chip and IEF groove of Solution Chip
2. Delta Marking

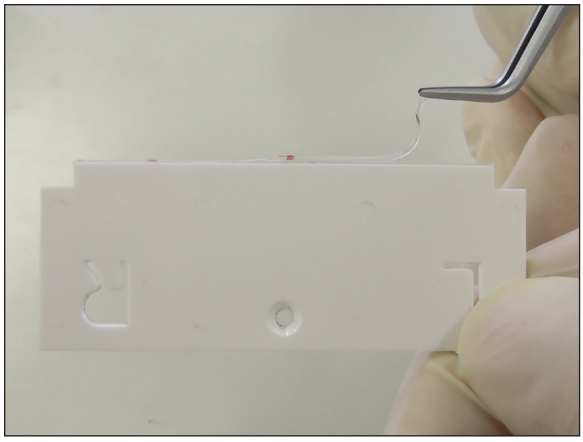
NOTE:

- Aligning the IEF portion of the Electrode Chip with the IEF groove of the Solution Chip will make it easy to assemble them.
- Check that the Electrode Chip fits into the Solution Chip securely.

CAUTION: Wet Electrode Chip may result in short circuit during IEF. Be sure the Chip is dry before setting it over the Chip set.

Install the IEF Chip

1. After the IEF Chip has reached room temperature, remove it from the pouch.
2. Remove the protective film from the IEF Chip with tweezers, taking care not to damage the gel.



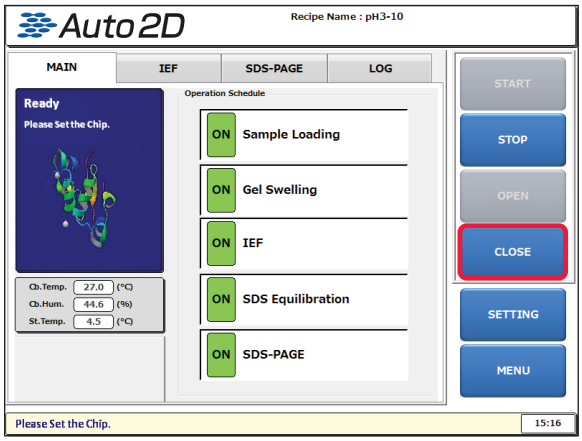
TIP: If the removal of the film is difficult, attach an adhesive tape to the film and pull it.

CAUTION: Be sure to check that the protective film has been removed completely otherwise the electrophoresis will not be performed properly.

3. Insert the IEF Chip in the slot on the Electrode Chip such that "L" and "R" marking locate at left and right, respectively, when viewed from the front.

Close the Tray

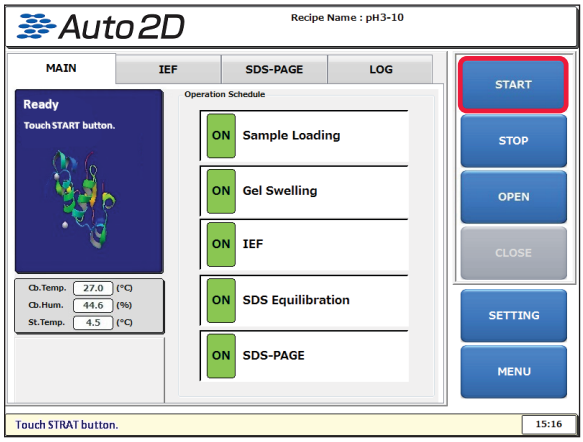
1. Check that Solution, Electrode and IEF Chips have been assembled properly and all the reagents have been applied.
2. Touch the [CLOSE] button on Main Menu. The Chip tray will be loaded into the Auto2D® Device automatically.



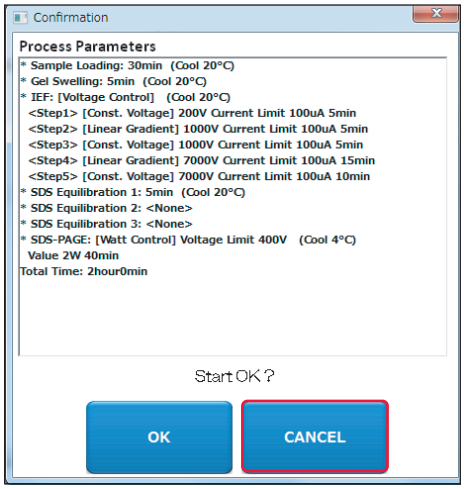
Steps of Operation

Start Electrophoresis

1. Touch [START] button on Main Menu.



2. Process Parameters Confirmation window will be displayed. Check each listed item carefully and touch [OK] button if all are OK. Electrophoresis will start automatically.
3. Press [CANCEL] button to change the process parameters or recipe.



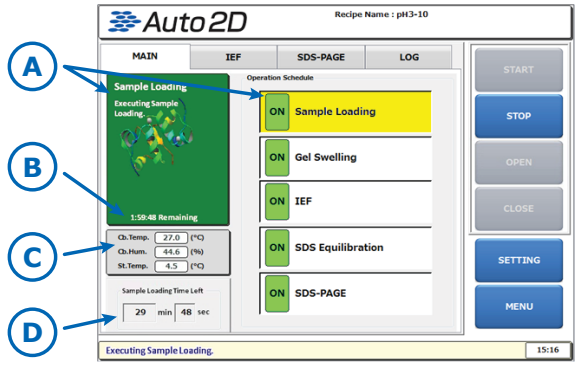
NOTE

- All data of voltage, current, temperature, and humidity can be monitored and saved in the SSD as CSV files.
- It takes about 100 minutes from start to finish of the electrophoresis process with the default protocol.

Screens during Electrophoresis

You can touch each tab to display a real time graph of voltage and current values for each process.

MAIN Tab



A. Current Process

B. Electrophoresis Remaining Time

C. Electrophoresis Chamber Data

D. Current Process Time Left

Electrophoresis Chamber Data

Cb. Temp.: Chamber Internal Temperature

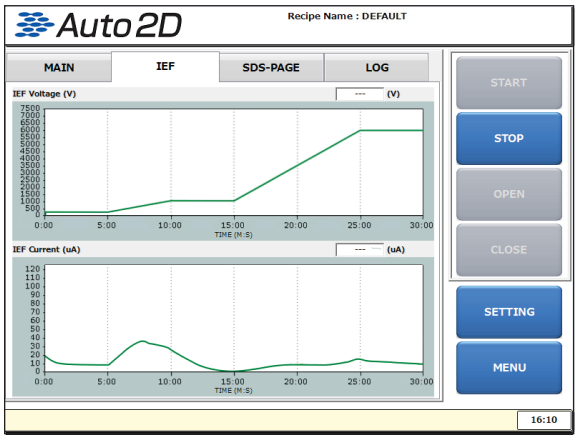
Cb. Hum.: Chamber Internal Humidity

St. Temp.: Stage Surface Temperature

CAUTION: The Humidity display turns red when the humidity drops to 40% or lower. Electrophoresis results are negatively impacted when the humidity is low and may cause evaporation of the reagents.

IEF Tab

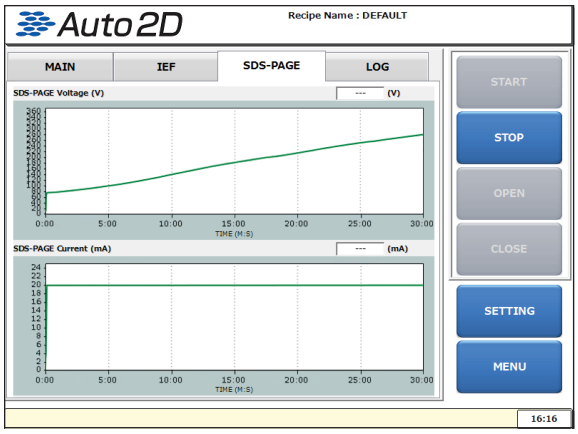
The IEF Voltage and IEF Current graphs can be checked.



NOTE: Data of IEF voltage, current, temperature and humidity of chamber can be saved in the SSD as a CSV file.

SDS-PAGE Tab

The SDS-PAGE Voltage and SDS-PAGE Current graphs can be checked.

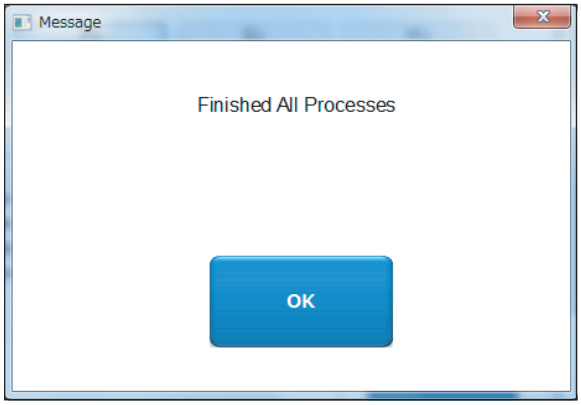


NOTE: The SDS-PAGE Voltage, SDS-PAGE Current, and Chamber Temperature and Humidity data can be saved in the SSD as a CSV file.

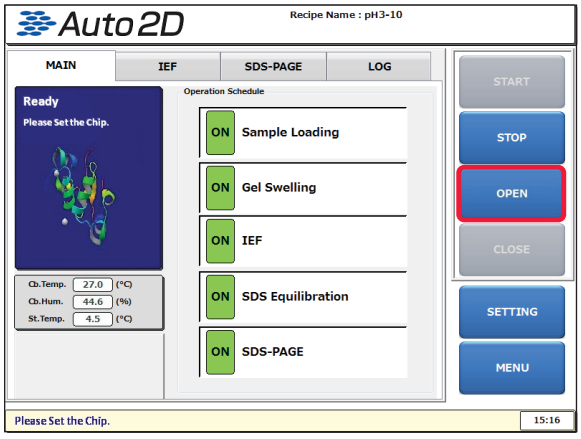
Remove PAGE Chip

(After Finishing Electrophoresis)

1. The following window will appear after the electrophoresis program has been completed.



2. Touch [OPEN] button on the Main Menu.



3. Remove the Electrode Chip.
4. Remove the Chip solution assembly. (Solution and IEF Chips on PAGE Chip).

   CAUTION: Be careful not to spill reagents when taking out the Chip solution assembly.
5. Dispose all the reagents and solutions remaining on PAGE and Solution Chips according to the applicable laws and regulations.

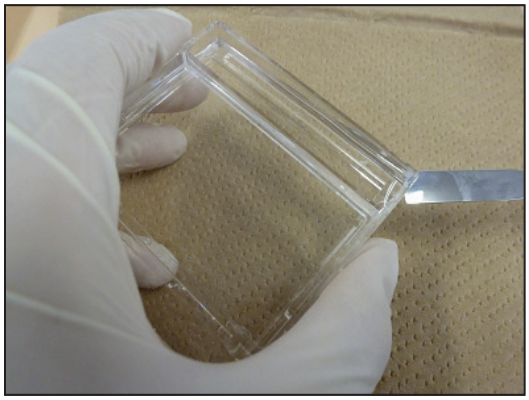
Remove Gel from PAGE Chip

When fluorescence imaging system (Either scanner type or camera type) is available and sample is labeled with a fluorescence dye prior to electrophoresis, the PAGE Chip can be scanned directly without removing the gel from the Chip. When other detection methods are used, remove the gel from the Chip following the instructions below.

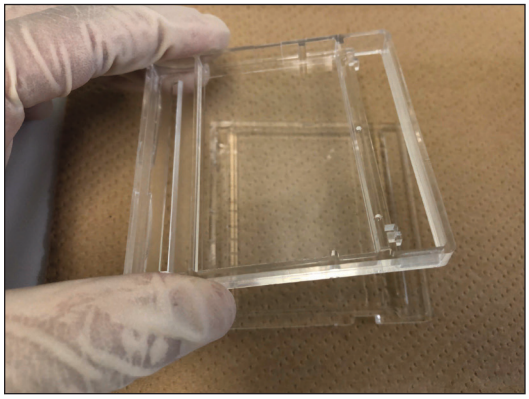
1. Disassemble PAGE Chip

   A. Insert the tip of a metal spatula between the top cover and Chip body at the 4 corners of the PAGE Chip and slightly twist to separate. (Noise will be heard at the time of separation.)

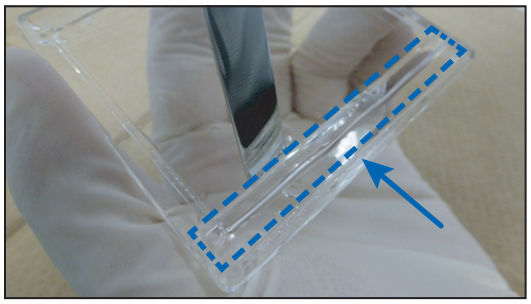
   CAUTION: Be careful not to damage the gel inside the PAGE Chip with the spatula.



   B. Slowly separate the top cover from the Chip body while paying attention not to tear or damage the gel.



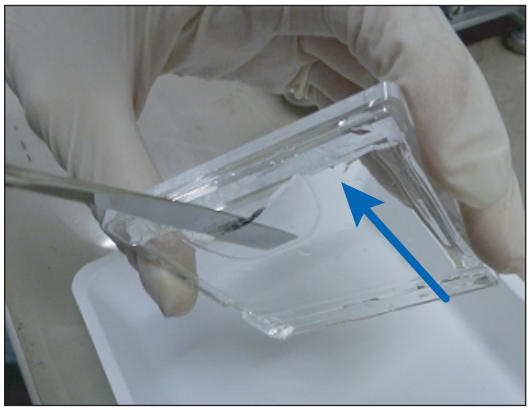
2. Remove excess gel.



   Excess gel at Anode side (Block shaped gel)

   Connection gel at Cathode side.

   NOTE: The excess gel lip must be removed before image analysis or blotting to a membrane.
3. Remove Remaining Gel from PAGE Chip



   Insert a metal spatula under the gel at anode side and remove the whole gel from the PAGE Chip body.

   Use spatula to cut a small piece from one corner of the gel. Record which corner and use this to orient the gel during staining and blotting to a membrane.

   TIP: Always remove the gel starting from the anode side to avoid damaging the separation area.
4. Proceed to gel staining or other analysis process.

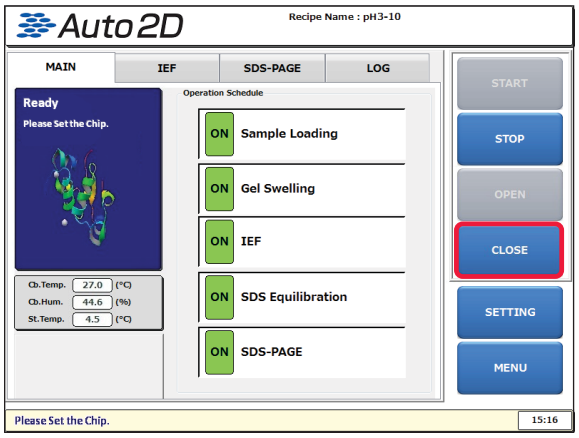
Cleaning Electrode Chip

(After finishing electrophoresis)

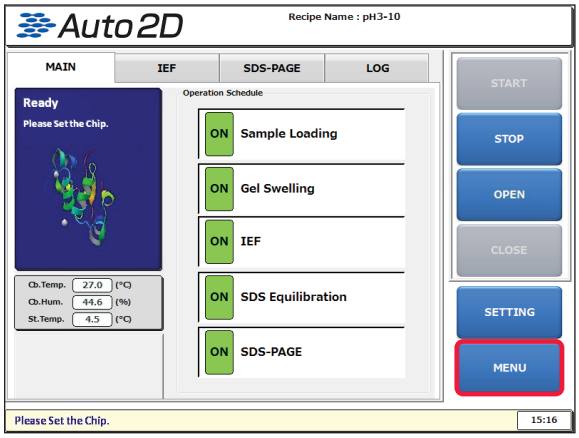
Clean the Electrode Chip with running water and then distilled water right after the use and allow it to air dry.

Turn off the Auto2D® Device

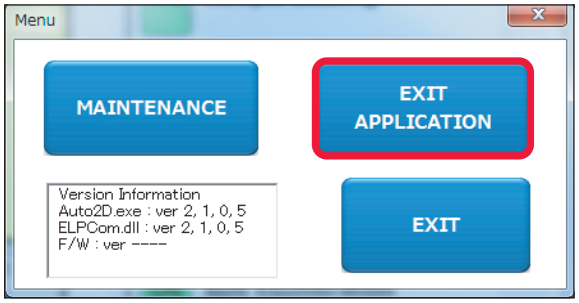
1. Touch the [CLOSE] button on the Main Menu to restore the Chip tray within the main body.



2. Touch the [MENU] button on the Main Menu. Menu window will pop up.



3. Touch the [EXIT APPLICATION] button on the Menu window and then the [System/OS Shutdown] button. The screen will turn off.



4. Turn off the Power Switch on the back of the Auto2D® Device.

CAUTION: A fan within the Auto2D® Device continues to run until the power switch is turned off. Be sure to turn off the Power Switch.

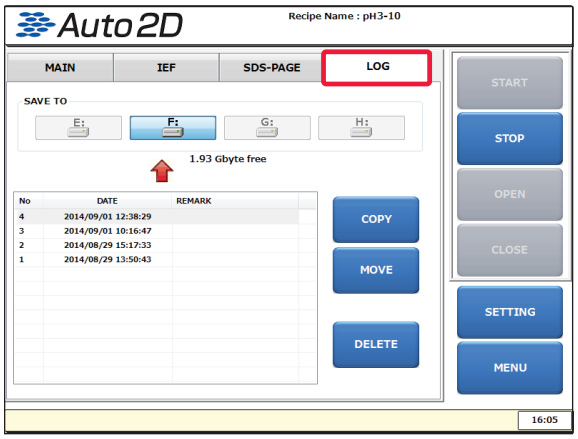
NOTE: If there is condensation inside device, leave tray opened for a while until the inside dries. When the machine is dry turn the power off.

CAUTION: In rare cases, the system will not boot up properly if not terminated properly. Please turn off according to these instructions to avoid persistent issues or damage to the Auto2D® Device. In the case the main power is on but the system does not start up, there is a reset switch on the lower right of the touch panel. For help contact Technical Service at SigmaAldrich.com/techservice.

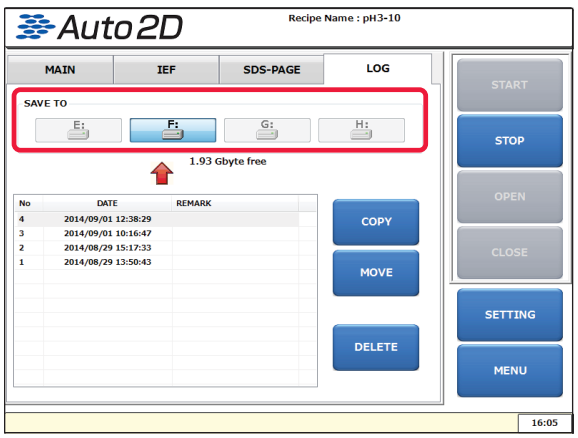
Saving Data

To Copy the Log

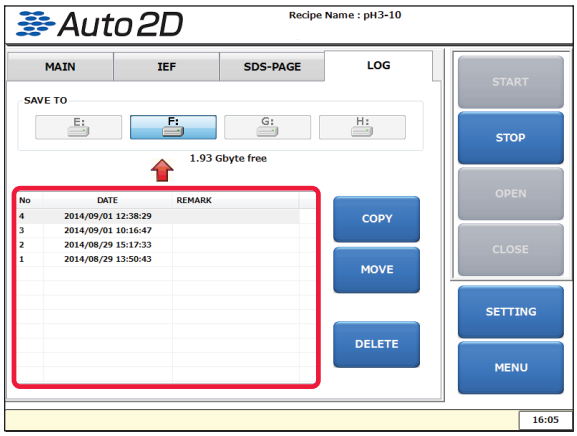
1. Connect an USB flash drive to the USB port on the right side of the Control Panel.
2. Touch the [LOG] tab on the Main Menu.



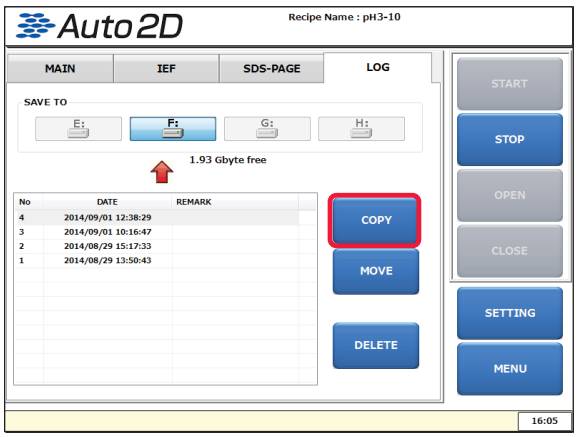
Select the drive to SAVE TO.



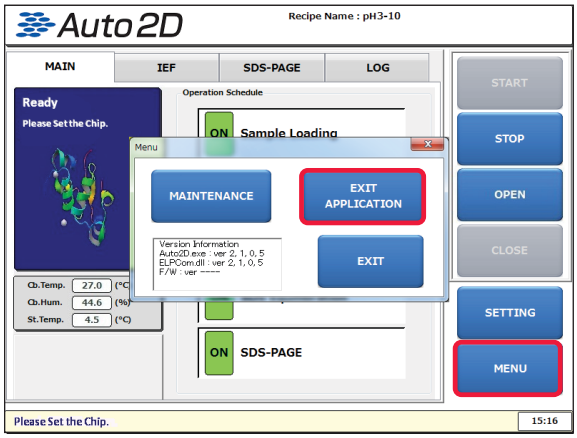
3. Touch and select a file to copy from the file list.



4. Touch the [COPY] button.
Confirmation window will pop up.
"Copied successfully." message will be displayed.



5. Touch the [MENU] button and then [EXIT APPLICATION] button. Touch the [System/OS Shutdown] button. Turn off the Power Switch of the Auto2D® Device.

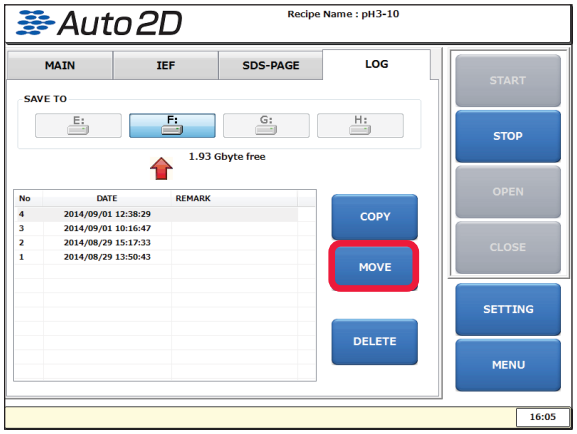


6. Disconnect the USB stick memory from the Auto2D® Device.

CAUTION: To avoid lost or damaged data, the Auto2D® Device must be completely powered off before removing USB flash drive.

Move the Log

1. Connect an USB flash drive to the USB port on the right side of the Control Panel.
2. Touch the [LOG] tab on the Main Menu.
3. Select the drive for SAVE TO.
4. Touch and select a file to move from the file list.
NOTE: Once a file is moved, the file is no longer accessible in the memory of the Auto2D® Device.
5. Touch the [MOVE] button.
Confirmation window will pop up.
"Moved successfully." message will be displayed.



6. Touch the [MENU] button and then [EXIT APPLICATION] button. Touch the [System/OS shutdown] button. Turn off the Power Switch of the Auto2D® Device.
7. Disconnect the USB stick memory from the Auto2D® Device.

CAUTION: To avoid lost or damaged data, the Auto2D® Device must be completely powered off before removing USB flash drive.

Auto2D® Original Recipes

Default

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 5 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000V, 5 minutes, Linear gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 6000V, 10 minutes, Linear gradient
5. 6000 V, 5 minutes, Constant Voltage
6. Not used
7. Not used

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Current

STEP 1
- Set Current: 20 mA
- Voltage Limit: 300 V
- Electrophoresis Time: 30 minutes

STEP 2 (Not used)
- Set Current: 20 mA
- Voltage Limit: 300 V
- Electrophoresis Time: 10 minutes

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 10 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 20 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE:
- The above standard recipe, "DEFAULT", is set as the factory default setting.
- The "DEFAULT" cannot be changed or modified.
- The "DEFAULT" does not use processes:
  - IEF STEP 6
  - IEF STEP 7
  - Equilibration Time 2
  - Equilibration Time 3
  - PAGE STEP 2

The red marked values are different from the "DEFAULT" recipe.

For IEF Chip, pH 3–10

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 5 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 7000 V, 15 minutes, Linear gradient
5. 7000 V, 20 minutes, Constant Voltage
6. Not used
7. Not used

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Power
- Set Power: 2 W
- Voltage Limit: 300 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE: This recipe does not use processes:
- IEF STEP 6
- IEF STEP 7
- Equilibration Time 2
- Equilibration Time 3
- PAGE STEP 2

The red marked values are different from the "DEFAULT" recipe.

For IEF Chip, pH 4–7

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 5 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 7000 V, 15 minutes, Linear gradient
5. 7000 V, 25 minutes, Constant Voltage
6. Not used
7. Not used

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Power
- Set Power: 2 W
- Voltage Limit: 300 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE: This recipe does not use processes:
- IEF STEP 6
- IEF STEP 7
- Equilibration Time 2
- Equilibration Time 3
- PAGE STEP 2

For IEF Chip, pH 4–5.5

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 5 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 5000 V, 15 minutes, Linear gradient
5. 5000 V, 20 minutes, Constant Voltage
6. Not used
7. Not used

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Power
- Set Power: 2 W
- Voltage Limit: 300 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE: This recipe does not use processes:
- IEF STEP 6
- IEF STEP 7
- Equilibration Time 2
- Equilibration Time 3
- PAGE STEP 2

The red marked values are different from the "DEFAULT" recipe.

For IEF Chip, pH 5–6.5

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 5 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 7000 V, 20 minutes, Linear gradient
5. 7000 V, 25 minutes, Constant Voltage
6. Not used
7. Not used

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Power
- Set Power: 2 W
- Voltage Limit: 300 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE: This recipe does not use processes:
- IEF STEP 6
- IEF STEP 7
- Equilibration Time 2
- Equilibration Time 3
- PAGE STEP 2

For IEF Chip, pH 6–10 or pH 7–10

Rehydration
- Sample Loading Time: 30 minutes
- Rehydration Time: 10 minutes

IEF
Current Limit: 100 µA
1. 200 V, 5 minutes, Constant Voltage
2. 1000 V, 5 minutes, Linear Gradient
3. 1000 V, 5 minutes, Constant Voltage
4. 4000 V, 20 minutes, Linear Gradient
5. 4000 V, 25 minutes, Constant Voltage
6. 8000 V, 10 minutes, Linear Gradient
7. 8000 V, 20 minutes, Constant Voltage

SDS Equilibrium
- Equilibration Time 1: 5 minutes
- Equilibration Time 2: Optional
- Equilibration Time 3: Optional

PAGE
Mode: Constant Power
- Set Power: 2 W
- Voltage Limit: 300 V
- Electrophoresis Time: 36 minutes (6.5%), 38 minutes (7.5%), 40 minutes (10%, 12.5%)

Temperature Setting
- Sample/Rehydration Temperature: 20 °C
- PAGE Temperature: 4 °C
- IEF Temperature: 20 °C
- Temperature at Power ON: 20 °C
- Equilibration 1 Temperature: 25 °C
- Equilibration 2 Temperature: 16 °C
- Equilibration 3 Temperature: 16 °C

NOTE: This recipe does not use processes:
- Equilibration Time 2
- Equilibration Time 3
- PAGE STEP 2

Troubleshooting

Do not attempt to repair the device yourself. Doing so will void the warranty.
Contact technical service at SigmaAldrich.com/techservice.

Symptom	General Cause	Corrective Action
No current flow	Insufficient Rehydration of the IEF Chip gel during IEF.	Set the Rehydration time longer so that the entire gel is being swelled.
	Insufficient Anode and/or Cathode buffer solutions during SDS-PAGE.	Add more Anode and/or Cathode buffer to the corresponding buffer groove until total volume is 4 mL or 4.5 mL and buffers reach the top of buffer grooves.
	Platinum wire of the electrode chip is damaged or cut.	Replace Electrode Chip.
Electrophoresis occurs too quickly during SDS-PAGE	Voltage is too high.	Check the voltage setting and lower it, as necessary.
Electrophoresis occurs too slowly during SDS-PAGE	High concentration of Anode and/or Cathode buffer solution.	Check the protocol for buffer solution and prepare the solutions at the correct concentration.
	Voltage is too low.	Check the voltage setting and increase it, as necessary.
	Sample has a high salt concentration.	Desalt the sample using buffer exchange with spin column, perform acetone precipitation or use a 2D cleaning kit.
	Solution leakage from the PAGE Chip.	Remove the Chip set and clean the spilled liquid, then replace it with new Chip set and restart.
Caused smiling effect	Gel temperature is too high during SDS-PAGE.	Check that water was loaded between the PAGE and Solution Chips. Check the SDS-PAGE temperature setting and lower it, as necessary.
	Uneven electric field between the electrodes.	Avoid air bubbles when applying Anode and Cathode buffer solutions to the PAGE Chip.
	Deteriorated Anode and/or Cathode buffer solution.	Use new buffer solution.
	Protein concentration is too high.	Reduce the amount of loaded proteins.
Spots cannot be observed	Protein concentration in the sample is too low.	Increase the protein concentration by using spin column.
	Insufficient solubilization of the proteins in the sample.	Change the composition of the Rehydration solution appropriately according to the proteins to be analyzed.
	Sample application time to the IEF chip was too short.	Set the Sample Loading Time longer.
	Low detection sensitivity.	Employ a method with high detection sensitivity.
Horizontal stripes across the gel	Insufficient sample solubilization.	Check that proteins have been fully solubilized. If not, review and change the composition of the Rehydration solution appropriately according to the proteins to be analyzed.
	Impurities in the sample.	Improve the sample preparation, remove impurities using a 2D Clean Up kit, desalt with spin column, or dilute the sample with Working Rehydration Solution.
	Protein concentration is too high.	Reduce the amount of loaded proteins or dilute the sample with Working Rehydration Solution.
	Proteins in the sample are aggregated or precipitated.	Vortex sample then centrifuge or filtrate the sample to remove the precipitates.
	Rehydration Solution or DTT are degraded.	Use new reagents.
	Sample was boiled or heated causing protein carbamylation.	Protein carbamylation is due to degradation of the Urea present in the rehydration solution. Avoid heating the sample during sample preparation.
	Insufficient focusing due to short IEF time.	Select a recipe with a longer IEF focusing time.

Symptom	General Cause	Corrective Action
Vertical stripes across the gel	SDS concentration in SDS equilibration buffer is too low.	Check the concentration of SDS in the SDS equilibriation buffer.
	Not enough SDS equilibriation.	Extend the equilibration time or use the Equilibration Buffer with Urea (7.5M Urea, 20 w/v% Glycerol, 125 mM Tris HCl pH 6.8, 50 mM DTT, 5 w/v% SDS, 0.01 w/v% BPB).
	Improper preparation of Anode and/or Cathode buffer solution.	Check composition and prepare fresh anode and cathode buffer solution.
	Proteins precipitated during the IEF process.	Check if ampholyte solution was added or double the concentration of ampholyte in the Working Rehydration Solution.
	Proteins in the sample are aggregated.	Before loading the sample, vortex and centrifuge it to remove the precipitants.
	High protein concentration.	Check the protein concentration, and if necessary dilute the sample with Working Rehydration Solution.
	IEF time is too long.	Select a recipe with a shorter time.
Streaks at high molecular weights	Low concentration of SDS.	Use our Reagent Kit.
Device cannot be turned on	Power failure.	Wait for power to be restored.
	Loose power cord contact.	Firmly push power cord into the outlet and into the back of the Auto2D® Device.
	Blown fuse.	Contact technical service.
Solutions spill inside the chamber	Device is not leveled or is unstable.	Level adjustment with the height adjustors at the bottom of the device.
Device overheating	Clogged filter of the fan.	Clean the filter with soft brush.
No display on the screen	Power switch of the device is in Off position.	Turn power switch on the back of the device to On position.
Electrophoresis is not completed	Insufficient rehydration of IEF Chip gel during IEF.	Increase the rehydration time.
	Insufficient volume of Anode and Cathode buffer during PAGE.	Apply appropriate volume of the buffer solution.
	Platinum wire of the electrode chip is damaged or cut.	Replace with new Electrode Chip or Electrode Chip Plus.
Clamp does not open or close	Possible computer error.	Restart the device by turning it Off and then On.
Arm does not move	Possible computer error.	Restart the device by turning it Off and then On.
Tray becomes stuck and does not open or close	Tray may be obstructed.	Remove obstruction.
	Electrode and Solution Chip improperly assembled before loading.	Abort the current run: Press [Abort] > [OK]. Put the instrument in maintence mode and open the tray: Press [MENU] > [MAINTENANCE] > [Tray (OPEN)].
Noise in the electric current graph	The Electrode Chip Plus may be dirty.	Clean the Chip gently with a soft brush and neutral detergent. Clean the elecrode portion gently with a soft brush or clean the electrode with an ultrasonic cleaning device. **CAUTION:** The platinum wire portion of the electrode is delicate. Be careful not to deform and damage it during the cleaning.
	The Electrode Chip Plus may be deteriorated.	Use a new Chip.
	The contact pin in Auto2D® Device may be deteriorated.	Contact Technical Service at SigmaAldrich.com/techservice for repair.

Symptom	General Cause	Corrective Action
Fluorescent label proteins in the gel do not seem to be labled	The sample might contain a high concentration of DTT or DNA that inhibits the labeling process.	Remove the inhibitor using 2D Clean Up kit, desalt with spin column, or improve sample preparation.
	The pH of sample is not appropriate.	Make sure the sample has a basic pH.
	The proteins are not completely denatured.	Dilute the sample more than 2 times with Rehydration Buffer.
The 2D patterns have poor reproducibility	The sample contains a lot of charged impurities that limit the voltage when the applied current exceeds the set value.	Remove the impurities in the sample or reduce the sample volume.
	The sample is not completely dissolved or the sample amount is too high; IPG gel could be clogged with proteins or impurities.	Make sure the sample is completely dissolved, vortex for few seconds and centrifuge to remove any precipitants.
Vertical streaks in the 2D gel after silver staining	Some silver staining kits may be incompatible.	Try a different staining kit.

If the device needs repair service, contact technical service at SigmaAldrich.com/techservice. Provide the date the device was purchased and details of the problem. The product information on the Rating Label (back of the Auto2D® Device) will also be needed.

Error Messages

Error Message	Corrective action
EMG Stop Peltier Over Heat	Close the application software. The monitor will turn off. DO NOT turn off the power switch. Leave Auto2D® for 10 minutes or more to cool it down, then turn the power switch Off and On to restart the device. Shorten the time of the process in which cooling temperature is low or set cooling temperature higher. Check if there is anything obstructing the fan filter at the front bottom of the instrument. Be careful not to damage the filter and fan.
Axis EMG Stop Z-Axis ALARM during Chip grip process	For Auto2D® Plus mode: Confirm that the Chips are for Auto2D® Plus mode and the Chips are set correctly. Access "Initialization:" Touch [MENU] > [MAINTENANCE] > [Initialization] > [Chip Release to Start Pos] > [Home position]. Double click or double touch [STOP] button to clear the error status.
Axis EMG Stop Z-Axis ALARM during SDS-PAGE process	Confirm that transparent cap on the cathode side of the PAGE Chip was removed. Access "Initialization:" Touch [MENU] > [MAINTENANCE] > [Initialization] > [Chip Release to Start Pos] > [Home position]. Double click or double touch [STOP] button to clear the error status.
Axis EMG Stop Z-Axis ALARM [Servo Error]	Double click or double touch [STOP] button to clear the error status. Remove the Chips from tray. Touch [MENU] > [EXIT APPLICTION] > [System/OS Shutdown] buttons. Turn off the power switch of Auto2D®, disconnect the power cord from the outlet and do NOT use the device. Please contact Technical Service at SigmaAldrich.com/techservice for repair.
Axis EMG Stop Y-Axis ALARM: Pos. Limit (+)	Access "Initialization:" Touch [MENU] > [MAINTENANCE] > [Initialization] > [Chip Release to Start Pos] > [Home position] buttons. Double click or double touch [STOP] button to clear the error message.
Chip Grip Process Y-Axis Positioning Timeout	Access "Initialization:" Touch [MENU] > [MAINTENANCE] > [Initialization] buttons. Double click [STOP] button to clear the error message.

Error Message	Corrective action
Initialization Z (or Y) -Axis Initialization Timeout	Double click or double touch [STOP] button to clear the error status. Remove the Chips from tray. Touch [MENU] > [EXIT APPLICTION] > [System/OS Shutdown] buttons. Turn off the power switch of Auto2D®, disconnect the power cord from the outlet and do NOT use the device. Please contact Technical Service at SigmaAldrich.com/techservice for repair.
Voltage Error Sample loading process	Select [Abort] button as good result is unlikely. The following are possible causes. • Sample: Improve sample preparation. The sample contains a lot of charged impurities. If the sample was prepared by acetone precipitate method or TCA/acetone precipitate method, residual acetone is likely to cause this error. Open the tube during sample preparation to evaporate the TCA or acetone. • Electrode Chip: Replace with new Electrode Chip. Wash the Electrode Chip with a brush if it is dirty. • Auto2D® Device: Please contact Technical Service at SigmaAldrich.com/techservice for repair.
Voltage Error IEF Process	If this error occurred during the final step of IEF, you may get good pattern. Otherwise, touch [Abort]. Impurities in the sample can overload the IPG gel. Improve sample preparation, use the 2-D Clean-Up Kit, or dilute the sample with Working Rehydration Solution. The Electrode Chip Plus or the contact pins in the Auto2D® Device may be deteriorated. Use a new Electrode Chip Plus or request maintenance of the Auto2D® Device.
Chip Grip Process Failed in Chip Grip	Ensure the tray is fully retracted into the instrument. Check that there are no obstructions or debris within the main body of the instrument which prevents smooth movement of the tray.
Tray Close Failed in Tray Close	Open the tray and confirm that the Chips are set correctly. Auto2D® Plus: Confirm that the transparent cap of the PAGE Chip is removed. Touch [Abort] > [OK] > [MENU] > [MAINTENANCE] > [Tray(OPEN)] When the error occurs even after resetting the Chips correctly, please contact Technical Service at SigmaAldrich.com/techservice for repair.
<ERROR> Check Communication Cable	Check the cables between the main body and control panel for any abnormalities. Restart the system and wait for about 20 seconds until connection is established. When the error occurs even after restarting, please contact Technical Service at SigmaAldrich.com/techservice for repair.
<WARNING> Chamber Humidity Low	Increase the humidity of the area around Auto2D® to an appropriate level with a humidifier, etc.
<ERROR> Current Over during Sample Loading process	The applied current has exceeded the set value. Improve sample preparation. The sample contains a lot of charged impurities such as salts, lipids and other oligos. If the current value goes down in the Sample Loading process, the 2D pattern is not affected. If the current does not go down, try removing the impurities with 2-D Clean-Up Kit or diluting the sample with Working Rehydration Solution.
<ERROR> Current Over during IEF process	There are impurities in the sample. Improve sample preparation, use the 2-D Clean-Up Kit, or dilute the sample with Working Rehydration Solution. IEF Chips pH 6–10 or pH 7–10 are being used. Recipes for these IEF Chips have a higher voltage (8 kV) than other recipes, so the gel thickness may become thinner on the basic end. Additionally, DTT migrates to the acidic end of the gel. This can cause the basic end to dry and the current value to rise sharply. In this case, substituting DTT with HED (1-hydroxyethyl disulfide) in the Working Rehydration Solution may solve this issue.

Frequently Asked Questions (FAQ)

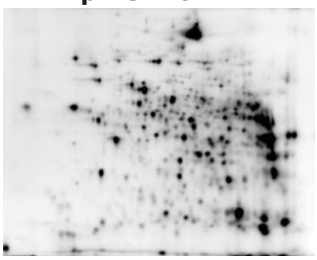
What is the difference between Auto2D® mode and Auto2D® Plus mode?

The main difference is in the sample loading. When using Auto2D® original mode, samples are loaded into the dry IPG gel by absorption prior to gel rehydration. Auto2D® Plus mode utilizes an electrical voltage to load protein samples into the rehydrated IPG gel. The result is a greater loading efficiency and shorter operation time when using Auto2D® Plus mode.

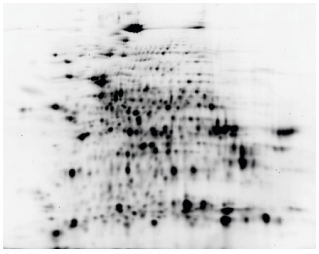
How do I select the correct IEF Chip?

- For a comprehensive overview of total protein distribution in the sample use pH 3–10 or pH 3–10 NL.
- For more detailed protein distribution in narrow pH range select an IEF chip pH 4–7 or pH 6–10. For a more reduced pH range, select pH 4–5.5, pH 5–6.5, or pH 7–10.

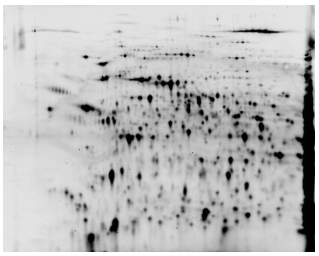
pH 3–10 NL



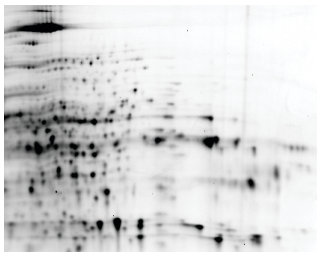
pH 3–10



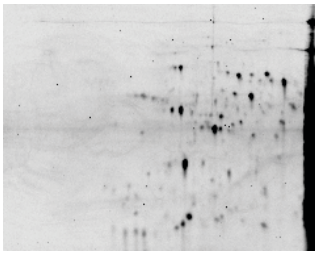
pH 4–7



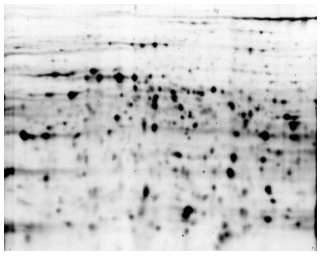
pH 6–10



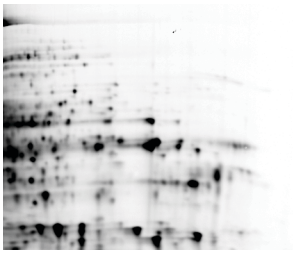
pH 4–5.5



pH 5–6.5



pH 7–10



Sample:
2 µg of mouse liver lysate labeled with cy5

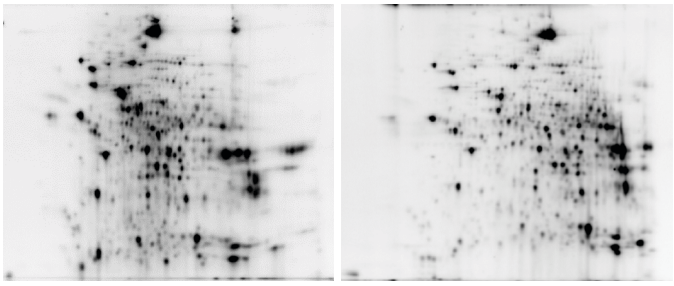
PAGE Chip:
10.0%

Reagent:
Tris-Glycine Reagent Kit

What is the difference between IEF Chip pH 3–10 and pH 3–10 NL?

NL stands for non-linear. IEF Chip pH 3–10 gives a linear overview of total protein distribution in a pH range of 3–10, while IEF chip pH 3–10 NL gives an overview of the sample but with wider resolution in the acidic region and narrower resolution in the basic region.

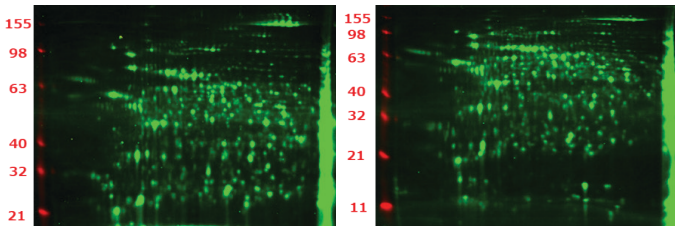
pH 3 **pH 10** **pH 3** **10 NL**



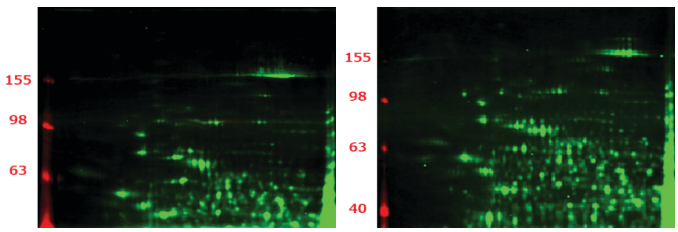
How do I select the correct PAGE Chip?

For comprehensive separation (from high to low molecular weight) select the 10% and 12.5% PAGE Chip. For separation of high molecular weight proteins select the 6 .5% or 7 .5% PAGE Chip. See figures below.

10% **12.5%**



6.5% **7.5%**



Sample: 2 µg of mouse liver lysate labeled with cy5
IEF Chip: pH 4–7
Reagent: Tris-Glycine Reagent Kit

How much protein should be loaded?

The recommended protein concentration will depend on the staining used, for example:

- Coomassie blue staining: 25 µg or more
- Fluorescent staining: 25 µg or less
- Silver staining: 10 µg or less
- Fluorescent pre-labeling: 3 µg or less

Which ampholyte should I use?

We recommend the following ampholytes.

For IEF Chip pH 3–10 NL:
IPG buffer pH 3–10 NL (Cytiva)

For IEF Chip pH 3–10:

- BioLyte® 3/10 100x (Bio-RAD) *Add 2 uL of BioLyte® to Rehydration solution.
- Mixture of ZOOM™ Carrier Ampholytes pH 3–10 and Pharmalyte® 3–10 (Cytiva), Ratio 1:1

For IEF Chip pH 4–7, pH 4–5.5 and pH 5–6.5:

- IPG buffer pH 4–7 (Cytiva)
- ZOOM™ Carrier Ampholytes pH 4–7 (Thermo Fisher Scientific)

For IEF Chip pH 6–10 and pH 7–10:
IPG buffer pH 6–11 (Cytiva)

Which IEF recipe should I use?

Use recipes with the same pH range as the IEF Chip. There are 3 recipes (L, M and S) for each pH range in Auto2D® Plus mode and one recipe for each pH range in Auto2D® original mode.

"M" recipes are the standard. Try these recipes first.
90 minutes; sample size of 1–50 µg.

"L" recipes
120 minutes; sample size of 30 µg or more

"S" recipes
60 minutes; sample size of 10 µg or less

What labeling dye can be used with the Auto2D® Electrophoresis system?

Samples can be pre-labeled using fluorescence dye reagent prior to electrophoresis. In 2-D electrophoresis, it is necessary to use dyes that do not affect the isoelectric point (total charge) of the proteins.

We recommend:

- Labeling of primary amine (N-term, Lys) using reagents with NHS-ester
  e.g.: Cy2/Cy3/Cy5 minimal labeling dye (Cytiva), IC3/5-OSu (Dojindo), Cyanine 3/5 NHS ester (Abcam)
- Labeling of Sulfhydryl group (-SH, S-S) using reagents with maleimide
  e.g.: Cy2/Cy3/Cy5 saturation labeling dye (Cytiva)

How do detergents used in sample preparation affect the 2DE?

Non-ionic detergents like DDM or Switterionic detergents like CHAPS can be used without affecting the 2DE.

The effect of other detergents on the 2DE depends on the type of the detergent and its concentration.

Ionic detergents like SDS with strong polarity commonly used during extraction can interfere with the IEF process by creating negative charge protein complexes. If the sample contains SDS or other strong ionic detergents, dilute it appropriately, perform buffer change or remove it using 2-D Clean Up kit.

What do you recommend for purification or desalting of samples?

We recommend 2D Clean Up kit for low-concentration samples as it can concentrate the samples.

A buffer exchange and sample concentration can be performed using Amicon Ultra 0.5ml or D-Tube dialyzer mini.

How do I prepare the sample after immunoprecipitation?

- If sample was eluted using the rehydration solution, it can be applied directly to the Auto 2D® without performing buffer exchange
- If sample was eluted using another buffer, change to Rehydration Solution with 2D Clean Up kit or desalt with spin column before applying it to Auto2D®.

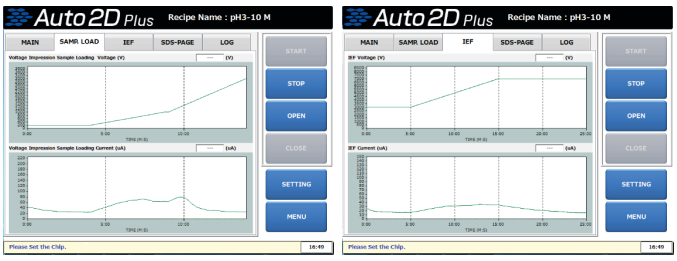
What information do the Voltage and Current graphs provide?

Voltage and current graphs indicate how successful the 2DE run was and provide tips to improve sample preparation.

For example, the current peak at 200 V results from the charge of small molecules like salts. The peaks at 1000 V result from the charge of medium molecules such as lipids and peptides, but the curret peak at high voltages comes from the charge or large molecules like proteins and nucleic acids.

If the current at final step of IEF process is stable below 50 µA, the separation is likely to be successful.

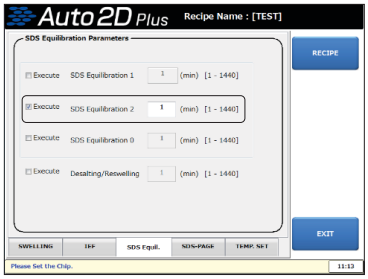
In the IEF, the current value gradually decreases and settles as the sample proteins have been focused on their individual isoelectric points.



Is it possible to perform alkylation of cysteine in the Auto2D® system?

Yes. Apply 700µL of Equilibration Buffer containing 2–3% iodoacetamide to the vacant Equilibration groove 2 of the solution Chip. And edit the recipe as follows.

1. Touch [SETTING] > [RECIPE] to move to the maintenance mode.
2. Load the recipe to edit.
3. Touch the "SDS Equilibration 2" checkbox on the "SDS Equil." Tab to activate it and set the time to 5 minutes.



4. Save as a new recipe.

Is it possible to perform non-reducing 2D conditions in the Auto 2D® system?

It is possible to perform non-reducing conditions by preparing the Working Rehydration Solution and/or Equilibration Buffer without DTT. If you perform 2nd dimension in the non-reducing environment, we recommend using Equilibration Buffer with Urea (7.5M Urea, 20w/v% Glycerol, 125mM Tris HCl pH 6.8, 5w/v% SDS, 0.01w/v% BPB).

Is it possible to perform Native conditions in the Auto 2D® system?

You can perform 2DE in Native conditions by combining Native-IEF and SDS-PAGE to separate both acidic and alkaline proteins. However, combining Native-IEF and Native-PAGE separates only acidic proteins.

How can I load MW markers in Auto2D Plus mode?

Auto2D Plus mode does not allow SDS-PAGE MW markers to be used. We recommend using proteins with a known molecular weight as a marker. The molecular weight can be estimated by adding the marker proteins to the sample. If you have a fluorescence imager, we recommend labeling the marker proteins with a different fluorescent dye from the sample. You can also separate the marker proteins and sample in each gel and compare them.

Product Ordering

Products can be ordered at SigmaAldrich.com.

	Qty	Catalogue Number
Auto2D® Device	1	BM-100

PAGE Chip

Gel Concentration (T%)	Qty	Catalogue Number
6.5%	10	BM-12065
7.5%	10	BM-12075
10.0%	10	BM-12100
12.5%	10	BM-12125

IEF Chip

pH Range	Qty	Catalogue Number
3–10	10	BM-113010
3–10 NL (non-linear)	10	BM-113010NL
4–7	10	BM-114070
4–5.5	10	BM-114055
5–6.5	10	BM-115065
6–10	10	BM-116010
7–10	10	BM-117010

Solution Chips

Description	Qty	Catalogue Number
Solution Chip	10	BM-1S
Solution Chip Plus	10	BM-1SP

Electrode Chips

Description	Qty	Catalogue Number
Electrode Chip	1	BM-1E
Electrode Chip Plus	1	BM-1EP

Reagent Kits

Description	Qty	Catalogue Number
Tris-Glycine Reagent Kit	1	BM-1RYSJ1
Tris-Tricine Reagent Kit	1	BM-1RYTJ1

NOTE: Tris-Glycine reagent kit is usually recommended. Tris-Tricine reagent kit is recommended when better resolution for low molecular weight proteins is needed.

Service Products

Service	Catalogue Number
Installation and Operations Qualification	BM-1IOQ
Validation and/or re-Operations Qualification	BM-1VAL

Notice

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