invitrogen

iBlot™ 2 Dry Blotting System

USER GUIDE

For dry, electroblotting of proteins from mini-, midi-, and E-PAGE™ gels

Catalog Number IB21001

Publication Number MAN0009112

Revision E.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0009112

Revision	Date	Description
E.0	10 January 2019	Replacing pictures in the "Replacing electrical contacts" section. Converted to CCMS.
D.0	15 March 2017	Addition of detailed information on proper method of closing device lid.

Contents

1 Product information

Product contents

Types of products

This manual is supplied with the iBlot™ 2 Gel Transfer Device (Cat. no. IB21001).

iBlot™ 2 Gel Transfer Device contents

The contents of the iBlot™ 2 Gel Transfer Device are listed below. See "iBlot™ 2 Gel Transfer Device specifications" on page 55 for specifications and description of the iBlot™ 2 Gel Transfer Device.

Component	Quantity
iBlot™ 2 Gel Transfer Device	1
Blotting Roller	1
Stylus	1
Power Cord	1
Power Adapters (North America/Japan, Europe, UK)	1 set

Before starting

Before you begin using this product, or any installation or service operation, please read the following safety information. Attention to these warnings will help prevent personal injuries and damage to the products.

It is your responsibility to use the product in an appropriate manner. This product is designed for use solely in laboratory environments, and must not be used in any way that may cause personal injury or property damage.

You are responsible if the product is used for any intention other than its designated purpose or in disregard of Thermo Fisher Scientific instructions. Thermo Fisher Scientific shall assume no responsibility for such use of the product.

The product is used for its designated purpose if it is used in accordance with its product documentation and within its performance limits.

Using the product requires technical skills and a basic knowledge of English. It is therefore essential that only skilled and specialized staff or thoroughly trained personnel with the required skills be allowed to use the product.

Keep the basic safety instructions and the product documentation in a safe place and pass them on to the subsequent users.

Applicable local or national safety regulations and rules for the prevention of accidents must be observed in all work performed.

The iBlot™ 2 Gel Transfer Device complies with the TUV Rhineland North America Inc. safety requirements, part 15 of the FCC rules, and the European Community Safety requirements. Operation of the iBlot™ 2 Gel Transfer Device is subject to the conditions described in this manual.

Operation of the iBlot™ 2 Gel Transfer Device is subject to the following conditions:

- Indoor use
- Altitude below 2,000 meters
- Temperature range: 5 to 40°C
- Maximum relative humidity: 80% (maximum relative humidity 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C)
- Installation categories (over voltage categories) II; Pollution degree 2
- Mains supply voltage fluctuations not to exceed 10% of the nominal voltage (100–240 V, 50/60 Hz, 6.3 A)
- Mains plug is a disconnect device and must be easily accessible.
- Do not attempt to open the iBlot™ 2 Gel Transfer Device. To honor the warranty,iBlot™ 2 Gel Transfer Device can only be opened and serviced by Thermo Fisher Scientific.
- The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Thermo Fisher Scientific.
- The device must be connected to a mains socket outlet with protective earthing connections.
- Ventilation requirements: Room ventilation
- Do not use if device becomes cracked or broken in any area.

Installing the instrument

The product may be installed only under the conditions and in the positions specified by Thermo Fisher Scientific.

Following are the required operating position and conditions:

- Do not place the product in an area where it will be subject to vibration.
- Do not place the product on surfaces, vehicles, cabinets or tables that for reasons of weight or stability are unsuitable for this purpose.
- Do not place the product on heat-generating surface or near heat emitting devices such equipment racks or heaters. Verify that there is sufficient clearance between the product and any other system that may exhaust warm air.
- The product's ventilation should not be obstructed. If proper ventilation is not provided it can result in electric shock, fire and/or serious personal injury or death.
- The product is for indoor use only.
- Use only with suitably rated mains supply cord (having 3 conductors, min. 16 AWG or 1.5 mm2, min. 300V, Harmonized Type for Europe and UL Listed/CSA Certified for North America, with molded plug rated min. 10A).
- A tolerance of ±10% shall apply to the nominal input voltage and ±3% to the nominal frequency, over voltage category 2.
- Maximum operating altitude 2,000 m asl. Maximum transport altitude 4,500 m asl.

Service operation requirements

In the event of an equipment malfunction, it is the responsibility of the customer to report the need for service to Thermo Fisher Scientific or to one of the authorized agents. For service information, contact Technical Support.

Servicing of this device is to be performed by trained service personnel only.

Unpacking instructions

Upon receiving the instrument

Examine the unit carefully for any damage incurred during transit. File any damage claims with the carrier. The warranty does not cover in-transit damage.

Unpacking the iBlot™ 2 Gel Transfer Device

- Remove the roller and stylus from the Styrofoam packaging.
- Remove the device from the Styrofoam packaging.
- Remove the protective film from the touch screen.

iBlot™ 2 Gel Transfer Device

Front view

The front-top view showing various parts of the iBlot™ 2 Gel Transfer Device is shown below.

Rear view

A rear view showing various parts of the iBlot™ 2 Gel Transfer Device is shown below.

Side view

A side view showing various parts of the iBlot™ 2 Gel Transfer Device is shown below.

About the system

iBlot™ 2 Dry Blotting System

The iBlot™ 2 Dry Blotting System consists of the iBlot™ 2 Gel Transfer Device and associated iBlot™ 2 Transfer Stacks (sold separately). The iBlot™ 2 Gel Transfer Device has a unique design, which, in conjunction with the patented gel matrix technology of the iBlot™ 2 Transfer Stacks, results in a shortened distance between electrodes, high field strength, and high currents to reduce transfer times when blotting proteins onto membranes.

Western blotting of proteins from midi- or mini-sized polyacrylamide gels onto nitrocellulose or PVDF membranes within 7 minutes can be performed with iBlot™ 2 Transfer Stacks.

See the next page to understand how the iBlot™ 2 Dry Blotting System works and “Description of parts“ on page 12 for details on various parts of the system.

Features

- Pre-programmed (iBlot™ 2 Gel Transfer Device) with six Methods for transfer of proteins from various gel types in 7–8 minutes
- Built-in safety features in the device enhance user safety
- User-friendly iBlot™ 2 Gel Transfer Device design with an integrated power supply to avoid inconsistencies associated with the use of an external power supply
- Fast, reliable protein transfer using iBlot™ 2 Transfer Stacks with integrated nitrocellulose or PVDF transfer membranes for blotting without the need to prepare buffers
- Compatible for use with Bolt™ Bis-Tris PLUS™, NuPAGE™ Bis-Tris and Tris-Acetate, Tris-Glycine, Tricine (in mini- and midi gel formats), and E-PAGE™ gels

System components

iBlot™ 2 Gel Transfer Device

The iBlot™ 2 Gel Transfer Device is a self-contained blotting unit with integrated power supply used for fast, dry blotting of proteins. See "iBlot™ 2 Gel Transfer Device" on page 12 for details.

iBlot™ 2 Transfer Stacks

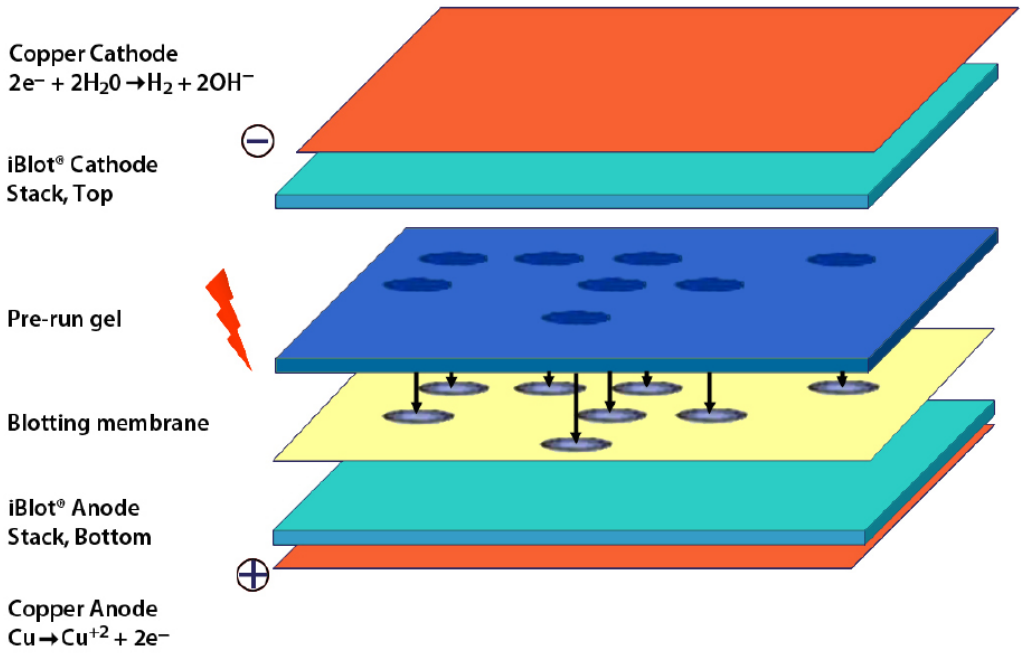
The iBlot™ 2 Transfer Stacks are disposable stacks that have integrated PVDF or nitrocellulose transfer membranes to perform dry blotting of proteins. Each iBlot™ 2 Transfer Stack contains a copper electrode and appropriate cathode and anode buffers in the gel matrix to allow fast, reliable transfer of proteins. See "iBlot™ 2 Transfer Stacks" on page 15 for details.

System overview

The iBlot™ 2 Dry Blotting System is based on the dry blotting concept, utilizing the unique, patented gel matrix technology developed for E-Gel™ and E-PAGE™ gels for the iBlot™ 2 Transfer Stacks.

The iBlot™ 2 Transfer Stack consists of a Bottom Stack and a Top Stack sandwiching a pre-run gel and a nitrocellulose (0.2 µm) or PVDF (0.2 µm) membrane. The iBlot™ 2 Transfer Stack is assembled with the blotting membrane on the anode side, and a pre-run gel on the cathode side.

Schematic of iBlot™ 2 Transfer Stack showing the flow of current



After the stack is assembled on the iBlot™ 2 Gel Transfer Device, and the appropriate Method is selected, the run is initiated. Complete transfer of proteins from the gel to the blotting membrane is accomplished in approximately 7–8 minutes. The rapid transfer without the need for external power supply or premade buffers is possible due to the following features of the iBlot™ 2 Dry Blotting System:

- The gel matrix of the Bottom and Top Stack incorporate the appropriate anode and cathode buffers to act as ion reservoirs. This format eliminates the need for premade buffers or soaked filter paper, and minimizes handling that can lead to inconsistent performance.
- The copper anode does not generate oxygen gas as a result of water electrolysis, resulting in increased transfer consistency. Conventional inert electrodes present in other blotting systems result in oxygen generation, which can result in blotting distortion.
- The design of the iBlot™ 2 Gel Transfer Device reduces the distance between the electrodes and the integrated power supply. This unique design combined with the gel matrix technology of iBlot™ 2 Transfer Stacks allows the system to generate high field strength and increase the transfer speed.

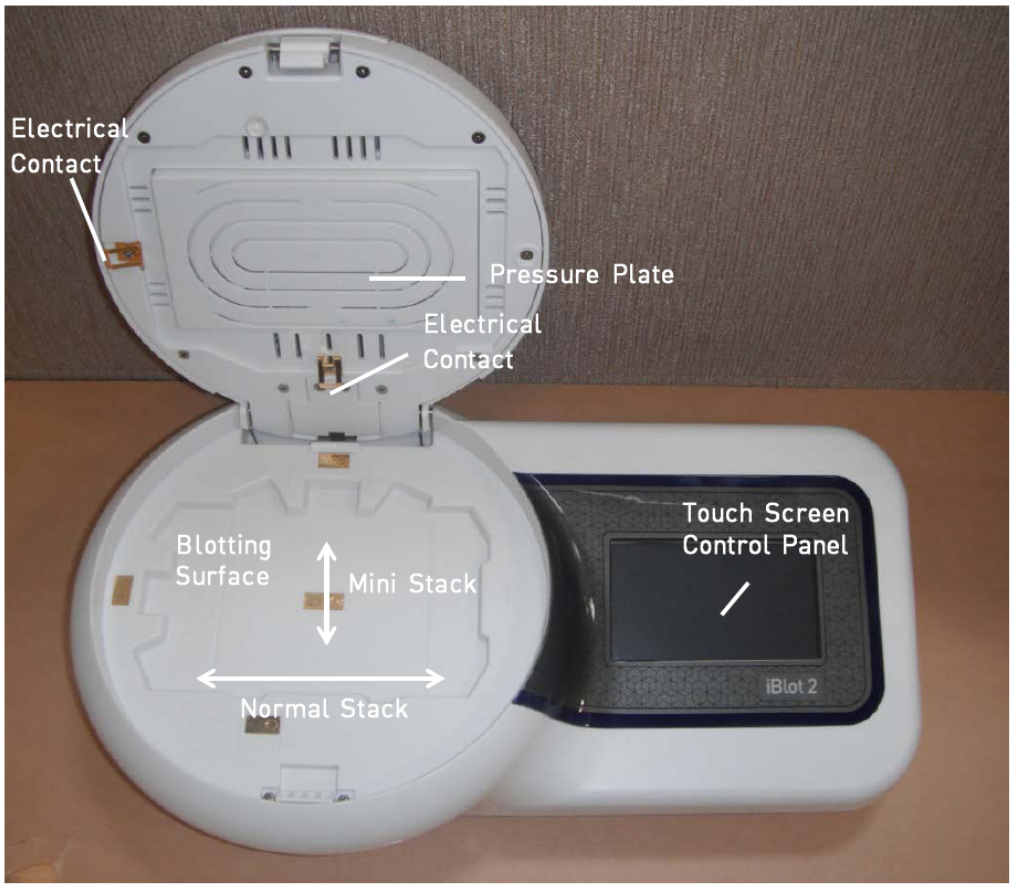
Description of parts

iBlot™ 2 Gel Transfer Device

The iBlot™ 2 Gel Transfer Device is a protein transfer device with an integrated power supply capable of producing currents up to 6.3 amp, and supplying voltage up to 25 V. Four printed circuit boards hold the electronic components required to process the systems logic unit, modify voltage and currents for display, and power the blotting process. A pre-installed firmware controls the parameters such as voltage and time, and allows selection of Methods (see "Description of methods" on page 23 for details on each Method).

IMPORTANT! When installing the iBlot™ 2 Gel Transfer Device, make sure it is placed on a level surface. Keep the area around the device clear to ensure proper ventilation of the unit. **For your safety:** Position the device properly such that the **Power** switch and the AC inlet located at the rear of the unit ("Rear view" on page 9) are easily accessible.

A top view of an open iBlot™ 2 Gel Transfer Device identifying various parts is shown below. See "iBlot™ 2 Gel Transfer Device" on page 8 for side and rear views of the device.



Blotting surface

The blotting surface is the area where the iBlot™ 2 Transfer Stacks containing the gel are placed to perform blotting. Alignment guides are used for proper orientation of normal and mini transfer stacks.

Lid

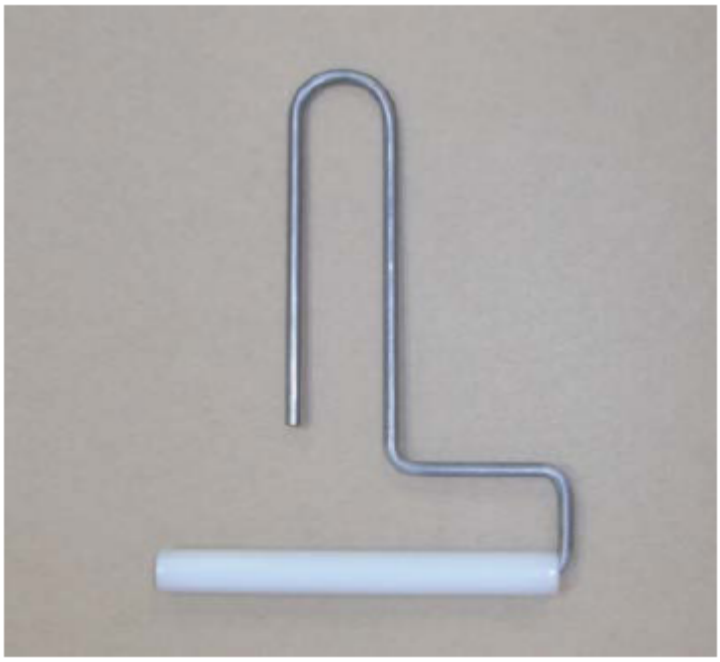
The lid of the iBlot™ 2 Gel Transfer Device contains ventilation holes to allow for proper ventilation of the unit during the run. The pressure plate exerts even pressure on the stack surface when the lid is closed.

Control panel

The Touch Screen Control Panel is a LCD display allowing the user to select Methods and control the device. See "Control panel of the iBlot™ 2 Gel Transfer Device" on page 18 for control panel details.

Blotting roller

The Blotting Roller is a plastic roller attached to a stainless steel handle (8.6 cm wide). The Blotting Roller is used to remove any air bubbles between the gel and blotting membrane during the assembly of the stacks and gel.



Stylus

A stylus is provided for use with the touch screen of the iBlot™ 2 Gel Transfer Device.

Power cord

The Power Cord connects to the iBlot™ 2 Gel Transfer Device on one end, and to a power adapter (for plugging into an AC electrical outlet) on the other.

IMPORTANT! Be sure that the AC power switch is in the Off position ("Rear view" on page 9) before attaching the power cord. Attach the power cord to the AC inlet of the device first, and then to the electrical outlet. Use only properly grounded AC outlets and power cords.

Power adapters

Power Adapters (North America/Japan, Europe, UK) are used to connect the power cord to an AC electrical outlet. Use the appropriate adapter for your geographical region.

iBlot™ 2 Transfer Stacks

The iBlot™ 2 Transfer Stacks are used to transfer proteins from gels onto nitrocellulose or PVDF membranes, and are available in Standard size for blotting E-PAGE™, midi-, or two mini gels, and Mini size for blotting one mini gel.

See "iBlot™ 2 Transfer Stack specifications" on page 55 for iBlot™ 2 Transfer Stack specifications.

Guidelines for iBlot™ 2 Transfer Stacks

- Store the iBlot™ 2 Transfer Stacks at room temperature. For best results, use the transfer stack before the expiration date printed on the package for each stack.
- Do not remove transfer stacks from bottom plastic tray. The plastic tray is a central part of the consumable. It maintains the current within the stacks and separates it from the rest of the device. The plastic tray also contains any liquid to allow easy clean-up.
- **Discard the iBlot™ 2 Transfer Stack after every use. Do not reuse the iBlot™ 2 Transfer Stack.**
- **Do not** use iBlot™ Transfer Stacks in the iBlot™ 2 Gel Transfer Device, or mix components between iBlot™ Transfer Stacks and iBlot™ 2 Transfer Stacks. Use iBlot™ 2 Transfer Stacks only for their designated application.

Note: The maximum voltage and current of the output to the gel stacks is 25 VDC and 6.3 Amp.

The following iBlot™ 2 Transfer Stacks are available at **thermofisher.com/iblot2** (see "iBlot™ 2 Transfer Stacks" on page 57 for ordering information).

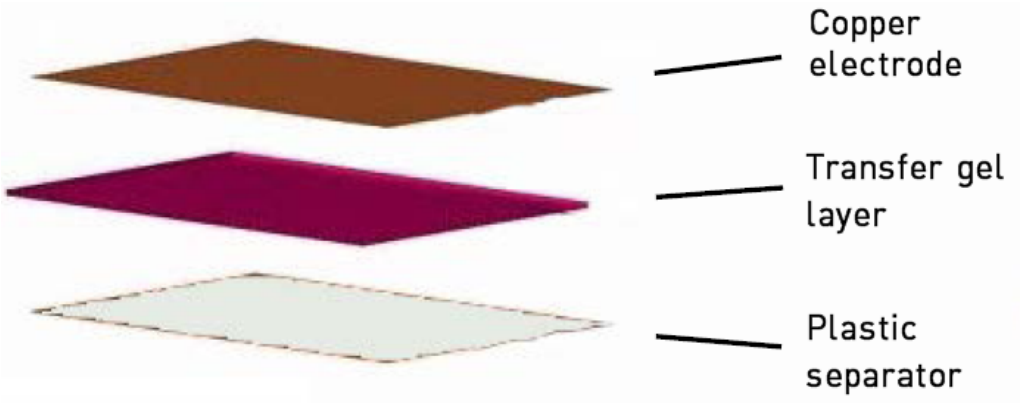
Product	Transfer Membrane	Cat. No.
iBlot™ 2 Regular Transfer Stacks	Nitrocellulose	IB23001
	PVDF	IB24001
iBlot™ 2 Mini Transfer Stacks	Nitrocellulose	IB23002
	PVDF	IB24002

Regular or Mini iBlot™ 2 Transfer Stacks come with the following components:

Component	Mini	Regular
iBlot™ 2 Transfer Stack	10	10
iBlot™ 2 Absorbent Pad, Regular	—	10
iBlot™ 2 Absorbent Pad, Mini	10	—
iBlot™ Filter Paper, Regular	—	10
iBlot™ Filter Paper, Mini	10	—

Top stack

The Top Stack is separated from the Bottom Stack by a white plastic separator, and contains a copper electrode and a transfer gel layer. The transfer gel layer acts as an ion reservoir and is composed of an optimized, proprietary gel composition.

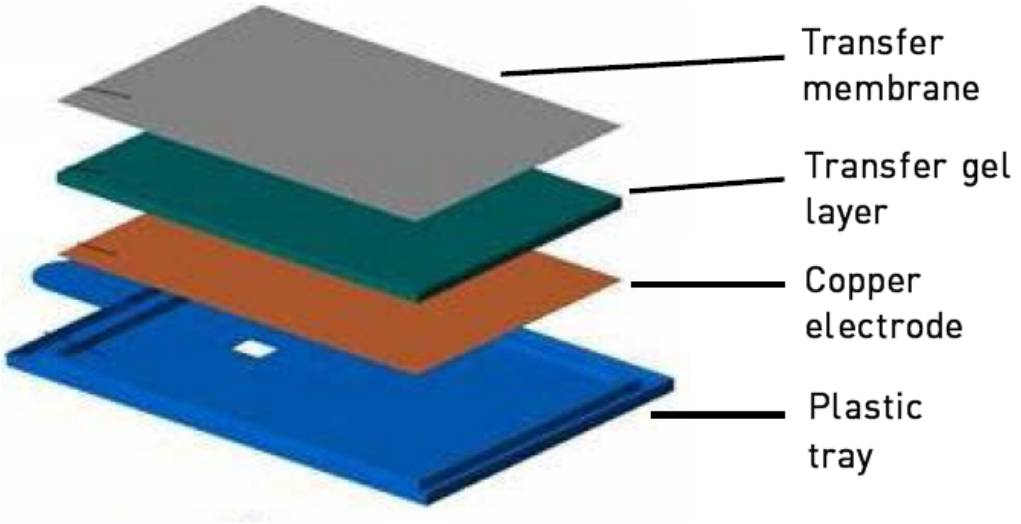


Bottom stack

The Bottom Stack contains a copper electrode, transfer gel layer, and a nitrocellulose (0.2 µm) or PVDF (0.2 µm) membrane for protein transfer. The transfer gel layer acts as an ion reservoir and is composed of an optimized, proprietary gel composition. The transparent plastic tray in which the iBlot™ 2 Transfer Stack is packaged serves as the support for assembling the transfer stacks with the gel.

The nitrocellulose (0.2 µm), and PVDF (0.2 µm) membranes **do not** require any pretreatment before use and minimize protein blow-through during the iBlot™ 2 blotting process.

Always use the Bottom Stack with the tray in the iBlot™ 2 Gel Transfer Device.



Transfer membrane

The iBlot™ 2 Transfer Stacks are assembled with the transfer membrane and are available with:

- Nitrocellulose membrane (0.2 µm)

  The nitrocellulose membrane is composed of 100% pure nitrocellulose to provide high-quality transfer. The membrane is compatible with commonly used detection methods such as staining, immunodetection, fluorescence, or

radiolabeling. The proteins bind to the membrane due to hydrophobic and electrostatic interactions. The protein binding capacity is 209 µg/cm^2.

- PVDF membrane (0.2 µm, low fluorescence)
  The PVDF membrane has higher binding capacity than nitrocellulose. **The PVDF membrane is preactivated and ready for use without any pretreatment with alcohol.** The membrane is compatible with commonly used detection methods such as staining, immunodetection, fluorescence, or radiolabeling. The proteins bind to the membrane due to hydrophobic interactions. The protein binding capacity is 240 µg/cm^2.

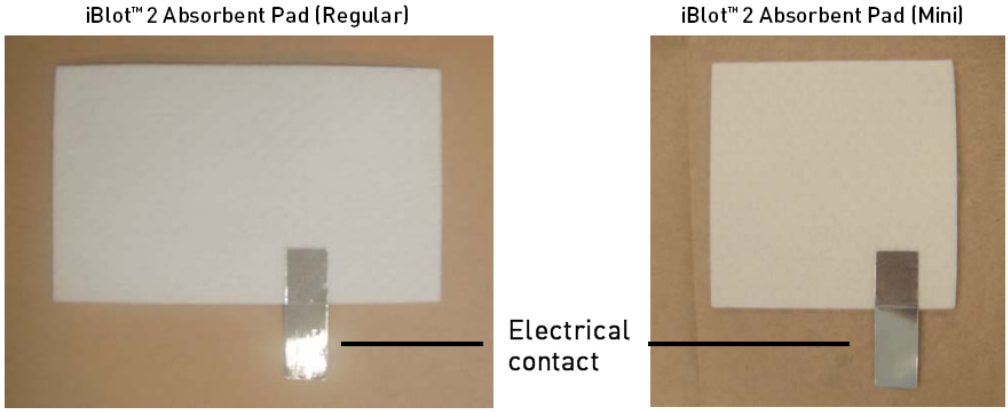
iBlot™ Filter Paper

The iBlot™ Filter Paper is used for blotting mini- or midi gels. The iBlot™ Filter Paper is placed on top of the pre-run gel before placing the Top Stack to protect the gel integrity during the blotting process. The iBlot™ Filter Paper is supplied in two sizes (see "iBlot™ 2 Transfer Stack specifications" on page 55 for dimensions) for efficient blotting of mini- and midi gels. Do not use the iBlot™ Filter Paper for blotting E-PAGE™ gels.

Note: Failure to use the iBlot™ Filter Paper during blotting of mini- or midi gels may result in high currents exceeding the current limit leading to a "High Current Error" during the run.

iBlot™ 2 absorbent pad

The iBlot™ 2 Absorbent Pad absorbs any excess liquid on the stacks formed during blotting and generates even pressure on the stack assembly. It is placed on top of the assembled iBlot™ 2 stack prior to transfer.



When properly assembled, the electrical contact of the iBlot™ 2 Absorbent Pad is aligned with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device to allow completion of the electrical circuit. See "iBlot™ 2 Transfer Stack specifications" on page 55 for dimensions of the iBlot™ 2 Absorbent Pad.

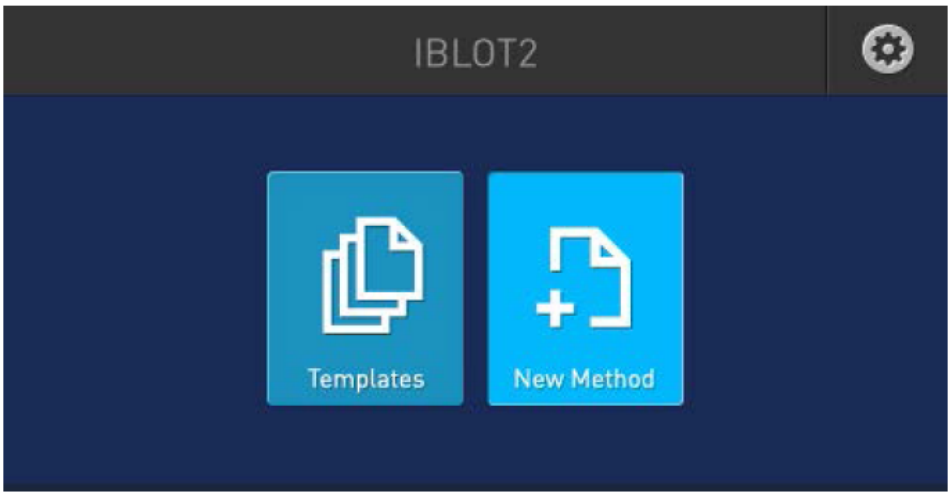
Discard the iBlot™ 2 Absorbent Pad after every use. Do not reuse the iBlot™ 2 Absorbent Pad.

Operating the iBlot™ 2 Gel Transfer Device

First time usage of the iBlot™ 2 Gel Transfer Device

The first time the iBlot™ 2 Gel Transfer Device is turned on, you will need to perform the following actions:

1. Configure the clock by touching the appropriate fields and entering the values. Touch **Enter** when done. Then Touch **Done** on the Date & Time screen.
2. The Tutorial screen is displayed. Take the tutorial if desired (the tutorial guides the user to assemble a stack and allows you to run the P0 Method if a stack is in the device. Otherwise hit **Cancel** to go to the Home Screen).
3. OR skip the tutorial to go to the Home Screen.



Control panel of the iBlot™ 2 Gel Transfer Device

The control panel is a touch screen display allowing:

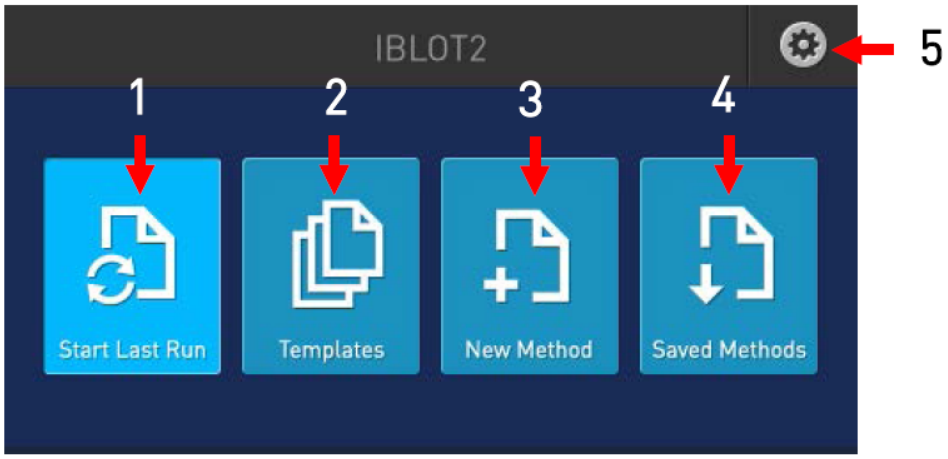
1. Running the Method that was last used on the device

   Note: This icon will not appear until after the first time the function is used.
2. Running Preset Templates (Default Methods)
3. Programming Custom Methods

   Note: For details on creating Methods, refer to "Creating custom methods" on page 36.
4. Running Custom Methods

   Note: This icon will not appear until after the first time the function is used.

5. Accessing the Options screen



iBlot™ 2 Gel Transfer Device options screen

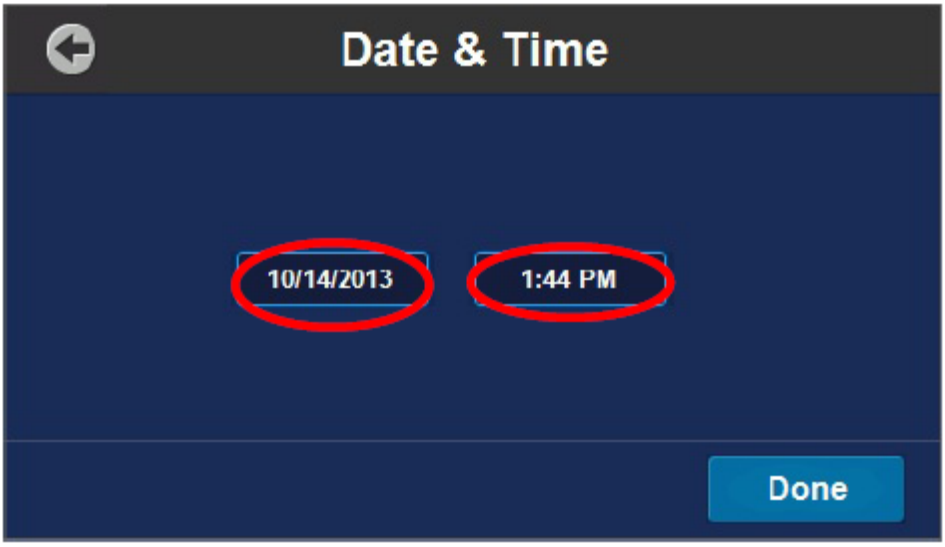
The Options screen allows the user to perform the following actions:

- Set date and time
- View a step-by-step tutorial for the iBlot™ 2 Gel Transfer Device
- Write logs to a USB device
- View application notes
- Upgrade firmware
- Reset the iBlot™ 2 Gel Transfer Device to factory settings

Setting date and time

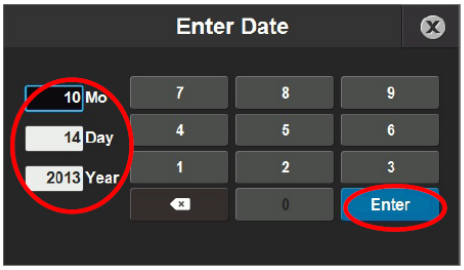
Touch **Date & Time** to access the Date & Time screen.

1. Touch the Date or Time field to access a keyboard for entering values.



2. Touch the fields that you want to change, and enter the appropriate value with the keyboard. Touch **Enter** when done.

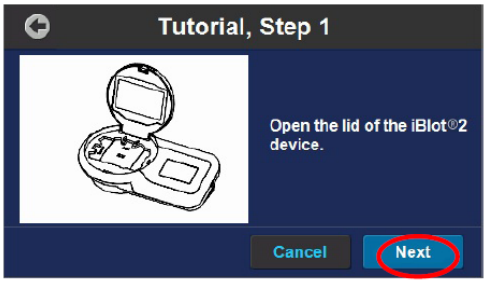
3. After entering the Date and Time, touch **Done** on the Date & Time screen to return to the Options screen.

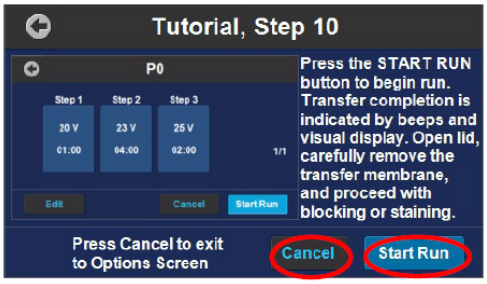


iBlot™ 2 Gel Transfer Device tutorial

The **Tutorial** button provides step-by-step instructions for assembling and running a transfer stack on the iBlot™ 2 Gel Transfer Device.

If following the tutorial to assemble a stack in real time, you can perform a run using the default P0 Method at step 10. Touch **Cancel** to return to the Options screen if you do not wish to perform a run.



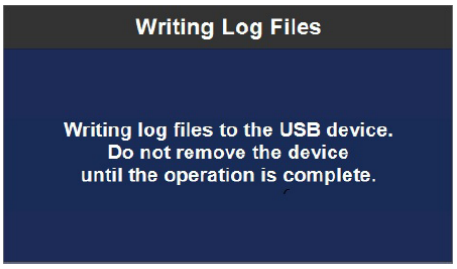


Writing logs to a USB storage device

A record of each run is kept by the iBlot™ 2 Gel Transfer Device, with voltage and current being tracked at 1-second intervals. This information can be downloaded to a USB storage device in ".csv" format.

1. Insert a USB storage device into the USB port (Type A) of the iBlot™ 2 Gel Transfer Device.
2. Touch **Write Logs to USB Device** on the Options screen.

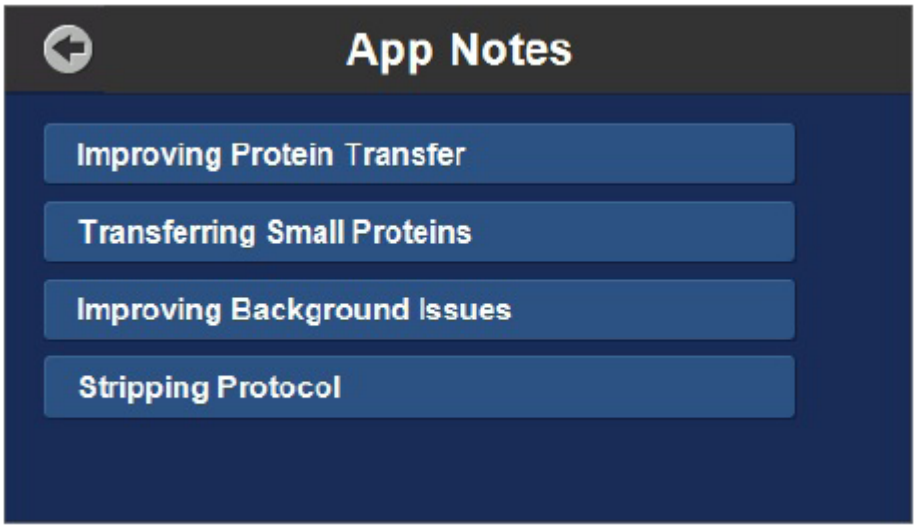
   Note: If you do not have a USB storage device in place, a prompt to insert a USB storage device will appear. Touch **OK** to start the transfer after inserting the USB storage device.
3. Do not remove the USB storage device until the Options screen is displayed again.



4. The files are saved to folders titled "iBlot™2_Logs" and "iBlot™2_ErrorLogs" on the USB storage device.

iBlot™ 2 Gel Transfer Device application notes

The **App Notes** button provides access to application notes for improving transfer under various conditions when using the iBlot™ 2 Gel Transfer Device.

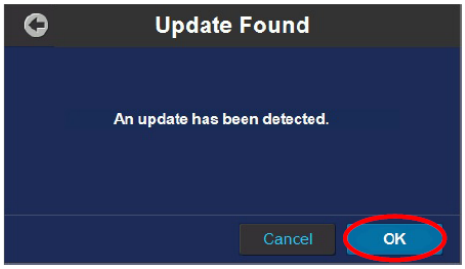


Upgrading iBlot™ 2 Gel Transfer Device firmware

The current firmware version on the iBlot™ 2 Gel Transfer Device is displayed on the **Firmware Version** button.

1. To update firmware, visit **thermofisher.com/iblot2**, and follow the instructions to download the latest firmware version to a USB storage device.
2. Insert the USB storage device with the new firmware version (".ib2" file) to be installed into the USB port (Type A) of the iBlot™ 2 Gel Transfer Device.
3. Turn on the iBlot™ 2 Gel Transfer Device and go to the Options screen.
4. Touch **Firmware Version**.

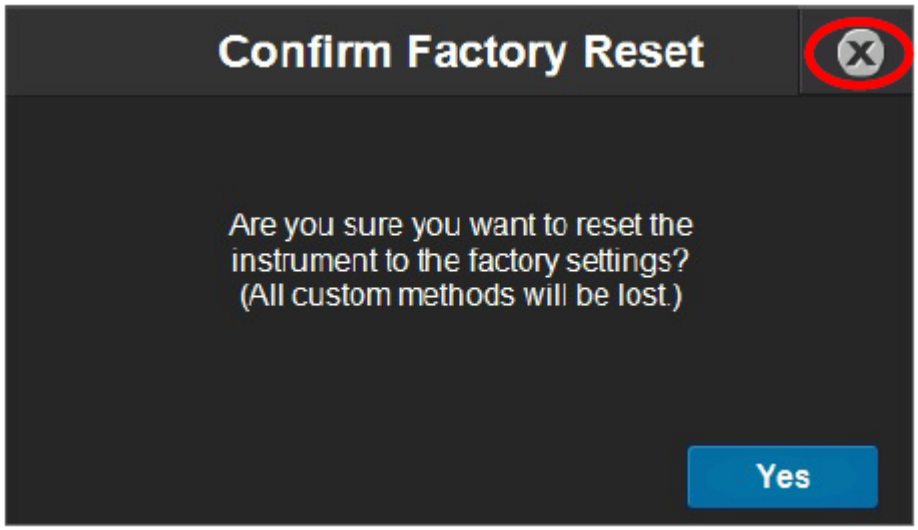
   Note: If you do not have a USB storage device in place, a prompt to insert a USB storage device will appear.
5. Touch **OK** to start the update. Do not remove the USB storage device until the Options screen is displayed again, showing the new firmware version.



Resetting the iBlot™ 2 Gel Transfer Device to factory settings

The **Reset to Factory Settings** button allows the iBlot™ 2 Gel Transfer Device to be restored to its original factory settings. If this option is selected, **all logs and custom methods will be deleted**.

If you do not wish to reset the iBlot™ 2 Gel Transfer Device, touch the cancel icon to return to the Options screen.



Description of methods

Methods

The iBlot™ 2 Gel Transfer Device is pre-programmed with six voltage Methods that allow blotting using different combinations of volts and time.

Method	Voltage	Default Run Time	Run Time Limit
P0	20 V for 1 minute 23 V for 4 minutes 25 V for 2 minutes	7 minutes	13 minutes
P1	25 V	6 minutes	10 minutes
P2	23 V	6 minutes	11 minutes
P3	20 V	7 minutes	13 minutes
P4	15 V	7 minutes	16 minutes
P5	10 V	7 minutes	25 minutes

The Default Run Time is the default time setting for a selected Method.

The Run Time Limit is the maximum recommended run time for a selected Method.

Recommended running parameters

The following parameters are recommended for transfer of proteins with molecular weights ranging from **30–150 kDa**.

Transfer Stack	Method	Volts	Run Time
Regular transfer stack (1 midi gel or two mini gels)	P0	20–25	7 minutes
Regular transfer stack (E-PAGE™ *48* or *96* gel)	P3	20	8 minutes
Mini transfer stack (one mini gel)	P0	20–25	7–8 minutes

The following parameters are recommended for transfer of proteins with molecular weights **>150 kDa**.

Transfer Stack	Method	Volts	Run Time
Regular transfer stack (1 midi gel or two mini gels)	P0, P3	20–25	8–10 minutes
Regular transfer stack (E-PAGE™ *48* or *96* gel)	P0, P3	20–25	8–10 minutes
Mini transfer stack (one mini gel)	P0, P3	20–25	8–10 minutes

You may need to optimize the blotting parameters (volts or time) based on your initial results (See "Optimizing blotting" on page 49 for details). This may include:

- Increasing or decreasing the transfer time.
- Performing an ethanol equilibration step prior to transfer can improve overall efficiency of protein transfer.

2 Protein transfer protocol

Experimental overview

Experimental outline

The table below outlines the experimental steps necessary to perform western blotting using the iBlot™ 2 Gel Transfer Device. For more details on each step, see indicated pages.

Step	Action	Page
1	Select Method for performing transfer.	"Selecting a method" on page 27
2	Remove the gel from the gel cassette.	"Removing the gel" on page 28
3	Assemble the iBlot™ 2 Transfer Stack with your protein gel for transfer using the iBlot™ 2 Gel Transfer Device.	"Assembling the iBlot™ 2 Transfer Stack" on page 29
4	Perform protein transfer using the selected Method.	"Performing blotting" on page 33
5	Disassemble the iBlot™ 2 Transfer Stack.	"Disassembling the iBlot™ 2 Transfer Stack" on page 34

General guidelines

To obtain the best results, follow these recommendations:

- Wear gloves at all times during the entire blotting procedure to prevent contamination of gels and membranes.
- Do not touch the membrane or gel with bare or gloved hands. This may contaminate the gel or membrane and interfere with further analysis. If you need to adjust the membrane, always use forceps.
- Avoid using expired iBlot™ 2 Transfer Stacks. Always use the transfer stacks before the specified expiration date printed on the package.
- Remove air bubbles as indicated in the protocol using the Blotting Roller supplied with the device.

- Do not trim the membrane or iBlot™ 2 Transfer Stacks to fit your gel size. See previous page for gel sizes that are compatible with iBlot™ 2 Gel Transfer Device. Note that iBlot™ 2 Mini Transfer Stacks are available for blotting mini gels ("iBlot™ 2 Transfer Stacks" on page 57). Maintain the membrane size identical to the transfer stacks to avoid direct contact between the top and bottom transfer stacks.
- Gently close lid after assembling transfer stacks to ensure metal contacts and transfer stacks do not shift out of position.
- Wipe down instrument and contacts after every use.

Recommended gel types

The gel types compatible for use with iBlot™ 2 Gel Transfer Device and iBlot™ 2 Transfer Stacks are listed below.

Gel Type	Size	iBlot™ 2 Transfer Stack
Midi gels (Novex™ Tris-Glycine PLUS™, NuPAGE™ Novex™ Bis-Tris, Tris-Acetate, or Tris-Glycine Midi gels, or equivalent)	13 cm (l) × 8.3 cm (w)1.0 mm thick	iBlot™ 2 Regular Transfer Stack
Mini gels (Bolt™ Bis-Tris PLUS™, NuPAGE™Bis-Tris or Tris-Acetate, Tricine, Tris-Glycine Gels, or equivalent)	8 cm (l) × 8 cm (w)1.0 or 1.5 mm thick	iBlot™ 2 Regular Transfer Stack, **or** iBlot™ 2 Mini Transfer Stack
E-PAGE™ *48* or *96* Gels	13.5 cm (l) × 10.8 cm (w)3.7 mm thick	iBlot™ 2 Regular Transfer Stack

Using the iBlot™ 2 Gel Transfer Device

Introduction

Instructions are provided in this section to assemble the iBlot™ 2 Gel Transfer Device for blotting mini-, midi-, or other gels.

Materials needed

You will need the following items:

- Pre-run mini- or midi gel containing your protein samples and standards
- iBlot™ 2 Regular Transfer Stack for blotting one midi gel, two mini gels, or one E-PAGE™ gel

  OR
- iBlot™ 2 Mini Transfer Stack for blotting one mini gel
- Blotting Roller supplied with the device

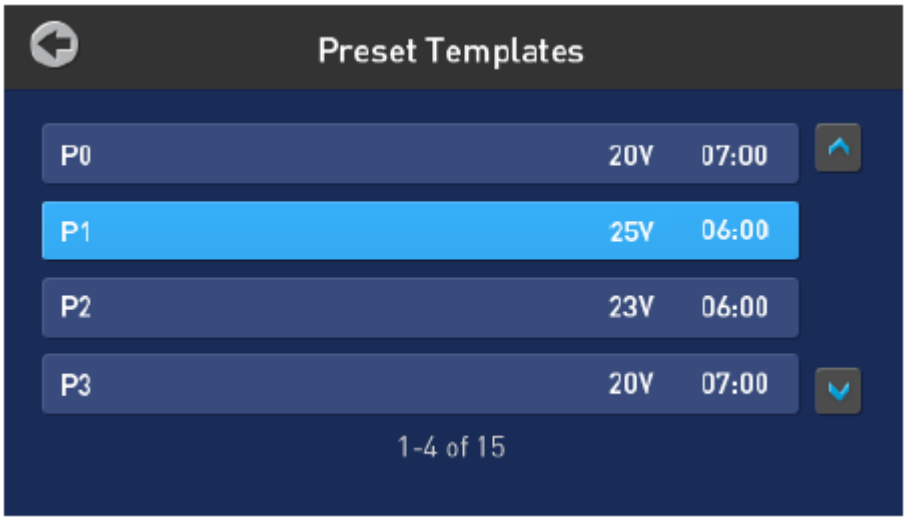
Selecting a method

Select the appropriate Method for your application on the iBlot™ 2 Gel Transfer Device prior to assembling an iBlot™ 2 Transfer Stack with your gel.

1. Touch the power switch at the rear of the device ("Rear view" on page 9) to turn **ON** the iBlot™ 2 Gel Transfer Device.

   The fan in the device begins to run and the digital display turns on.

2. Select the Method by touching:
   - **Templates** to access the desired Method (see "Control panel of the iBlot™ 2 Gel Transfer Device" on page 18)
   - **Saved Methods** to select a Custom Method (page)
   - **Start Last Run** to use the Method from the last run



Removing the gel

Remove the gel from the cassette for transfer after completion of electrophoresis as described below.

- Open the mini- or midi gel cassette using the Gel Knife by inserting the knife into the narrow gap between the two plates of the cassette. Push up and down gently on the handle of the knife to separate the plates. Upon opening the cassette, discard the plate without the gel and slowly remove the gel adhered to the other plate. For details on removing the gel, refer to the manual supplied with the mini- or midi gel.
- For other gel types, refer to the manufacturer recommendations to remove the gel from the cassette.

General guidelines

- There is generally no need for any pretreatment of the gel after electrophoresis, however, under certain circumstances, an equilibration step can improve results:
  - Equilibration of the gel in 20% ethanol (prepared in deionized water) for 5–10 minutes prior to performing blotting can improve the overall efficiency of transfer for high molecular weight proteins.
  - For **Novex™ Tris-Glycine PLUS™ Gels**, equilibration in 100 mL of deionized water or transfer buffer for 5 minutes prior to transfer may improve transfer of mid to small molecular weight proteins. This equilibration step can be omitted for optimal transfer of higher molecular weight proteins (>150kDa).
- The transfer membrane is supplied in a ready-to-use format in the stacks without any need for pretreatment. Do not treat the PVDF membrane with methanol as the PVDF membrane is preactivated prior to assembly with the transfer stack.
- You may blot E-PAGE™ gels using the blotting protocol with the Blotting Roller. If you wish to use the Blotting Roller for blotting E-PAGE™ gels be sure to:
  - Wash the E-PAGE™ gel briefly in deionized water prior to blotting to remove any small gel pieces attached to the gel.
  - Use the Blotting Roller all over the gel including **all well areas** to flatten any protrusions to ensure even transfer and efficient blotting.
- When placing an E-PAGE™ gel on the membrane, make sure the open wells face upwards, and that the bottom of the gel is in contact with the membrane.

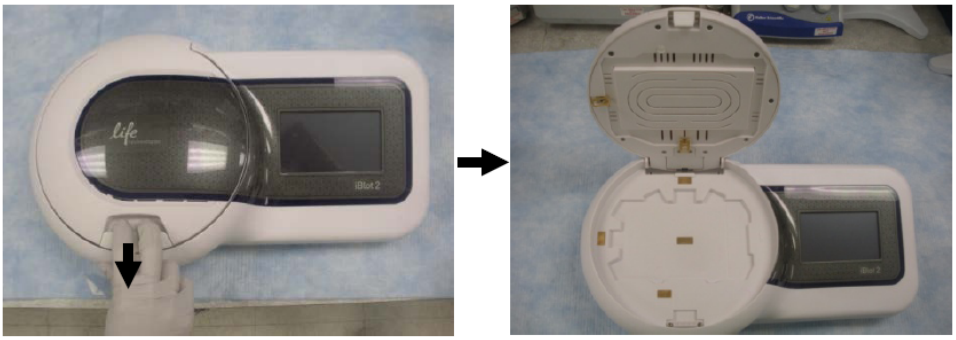
Use the appropriate iBlot™ 2 Transfer Stacks based on the gel that you are blotting. **Do not** trim the membrane or transfer stacks to fit the size of your gel, as the transfer quality is not affected if the pre-run gel is smaller than the transfer stack. Always maintain the membrane size identical to the transfer stacks to avoid accidental contact between the Bottom and Top Stacks.

See “Recommended gel types“ on page 26 for gel types compatible with the iBlot™ 2 Gel Transfer Device.

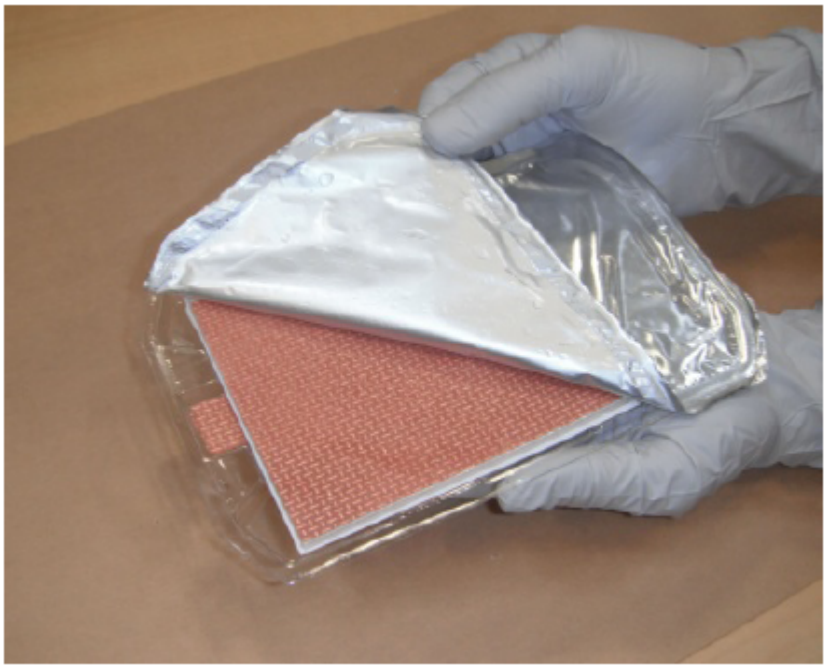
- Use the iBlot™ 2 **Regular** Transfer Stacks for blotting two mini gels or one midi gel
- Use the iBlot™ 2 **Mini** Transfer Stacks for blotting one mini gel.

Assembling the iBlot™ 2 Transfer Stack

1. Open the lid of the device using the latch. Ensure the blotting surface is clean.

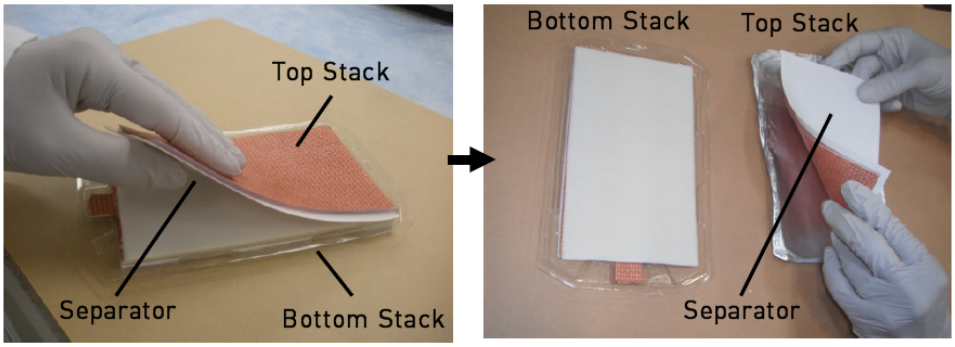


2. Unseal the iBlot™ 2 Transfer Stack.



3. Separate the Top Stack and set it to one side of the bench with the transfer gel layer facing up. **Keep the Bottom Stack in the transparent plastic tray.**

   Note: In some instances, the membrane may adhere to the separator. Make sure that the membrane is not stuck to the separator before proceeding to the next step. If the membrane is stuck on the separator, use forceps to remove the membrane and place it on top of the Bottom Stack.



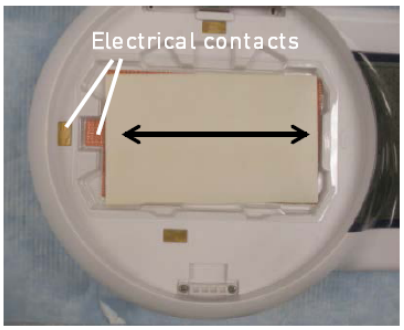
4. Place the Bottom Stack **with the plastic tray** directly on the blotting surface.

   Align the tray in the center of the blotting surface according to the type of iBlot™ 2 Transfer Stack being used.

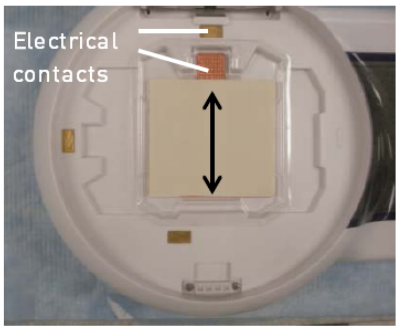
   Do not push trays fully to the top (Mini) or fully to the left (Regular) as this may elevate and twist the tray or interfere with the contacts.

   The electrical contacts on the tray should be aligned with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device.

Orientation for iBlot™ 2 **Regular** Transfer Stack



Orientation for iBlot™ 2 **Mini** Transfer Stack



5. Ensure there are no bubbles between the membrane and the transfer stack. Remove any trapped air bubbles using the Blotting Roller.

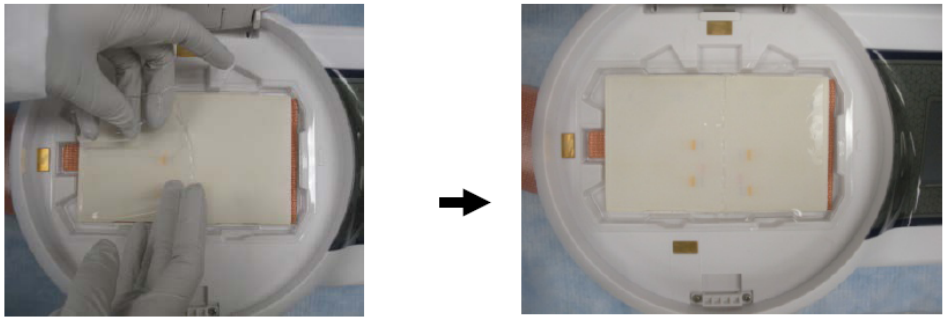
6. Open the gel cassette and immerse the pre-run gel briefly in deionized water (1–10 seconds) to facilitate easy positioning of the gel on top of the transfer membrane.

   Note: For Novex™ Tris-Glycine PLUS™ gels, rinse in 100 mL of deionized water for 5 minutes to improve transfer of mid to low molecular weight proteins (< 150 kDa).

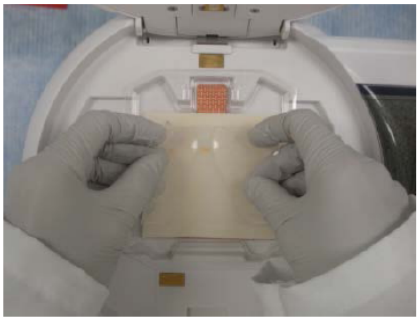
7. Shake off excess water, and place the pre-run gel on the transfer membrane of the Bottom stack as described:

8. 1 midi gel on an iBlot™ 2 Regular Transfer Stack
9. 2 mini gels (head-to-head) on an iBlot™ 2 Regular Transfer Stack
10. 1 mini gel on an iBlot™ 2 Mini Transfer Stack

Transferring two mini gels



Transferring one mini gel

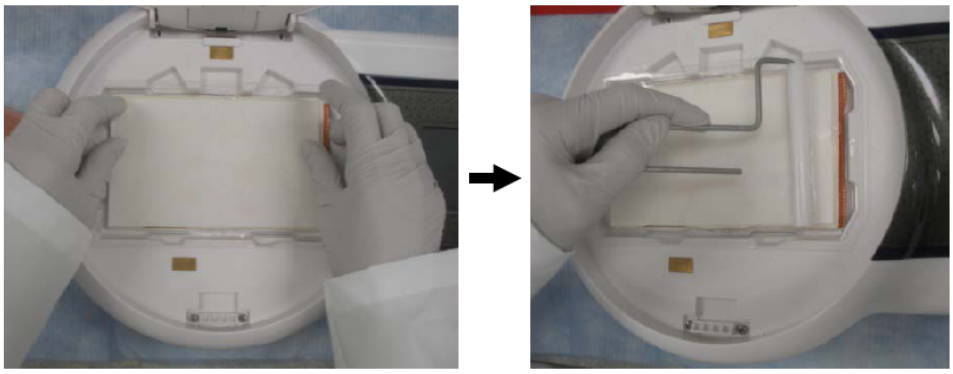


11. Use the Blotting Roller to remove any air bubbles between the gel and the membrane.

12. Soak the iBlot™ Filter Paper (use the appropriate filter paper for the size of the gel) in a clean container of deionized water. iBlot™ Filter Paper is included with each iBlot™ 2 Transfer Stacks.

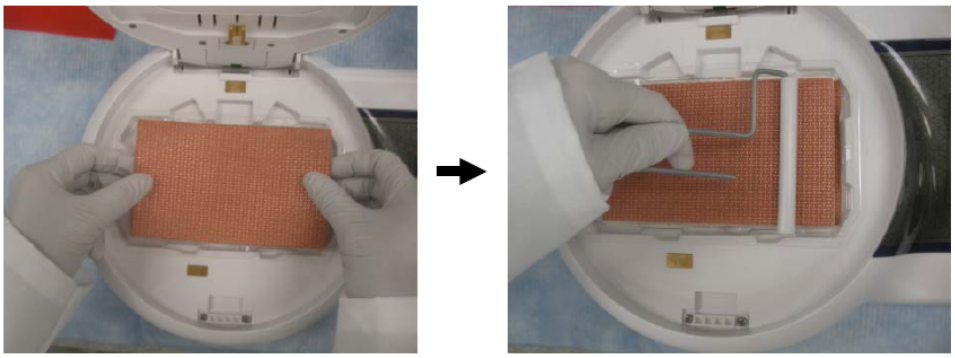
    Note: For E-PAGE™ gels, **there is no need** to use a filter paper.

13. Place the presoaked iBlot™ Filter Paper on the pre-run gel. Use the Blotting Roller to remove any air bubbles between the filter paper and gel.



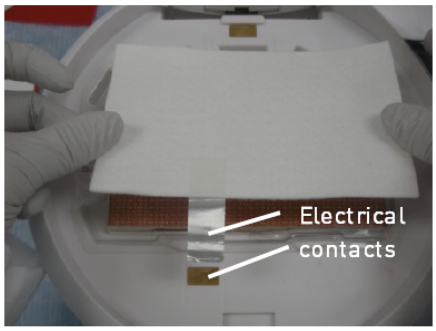
14. Remove and discard the white plastic separator from the Top stack.

15. Take the Top Stack from the bench and place it on top of the presoaked filter paper with the copper electrode facing up (and transfer gel layer facing down). Remove any air-bubbles using the Blotting Roller.

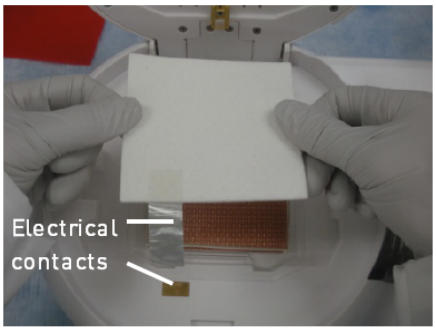


16. Place the iBlot™ 2 Absorbent Pad on top of the iBlot™ 2 Transfer Stack such that the electrical contacts are aligned with the corresponding electrical contacts on the blotting surface of the iBlot™ 2 Gel Transfer Device.

Orientation for iBlot™ 2 Absorbent Pad, **Regular**



Orientation for iBlot™ 2 Absorbent Pad, **Mini**



17. Use the Blotting Roller to flatten any protrusions in the transfer stack.

Performing blotting

After assembling the iBlot™ 2 Gel Transfer Stack, perform blotting as described below. Perform blotting within 10–15 minutes of assembling the stacks with the gel.

1. Gently close the iBlot™ 2 Gel Transfer Device lid by pressing down with two hands on the sides of the lid. Make sure the latch is secure. Do not forcibly push the lid when closing, because it can cause the transfer stack or metal contacts to shift out of position

2. Ensure that the correct Method is selected (“Recommended running parameters“ on page 23).

3. Touch the Start icon on the screen to begin the transfer.

4. At the end of the transfer, the current automatically shuts off and the iBlot™ 2 Gel Transfer Device signals the end of transfer with repeated beeping sounds, and a message on the digital display.

5. Touch the Done icon to stop the beeping.

6. Proceed to **Disassembling the iBlot™ 2 Transfer Stack**, "Disassembling the iBlot™ 2 Transfer Stack" on page 34.

Disassembling the iBlot™ 2 Transfer Stack

Introduction

Refer to the instructions below to disassemble the iBlot™ 2 Transfer Stack.

Procedure

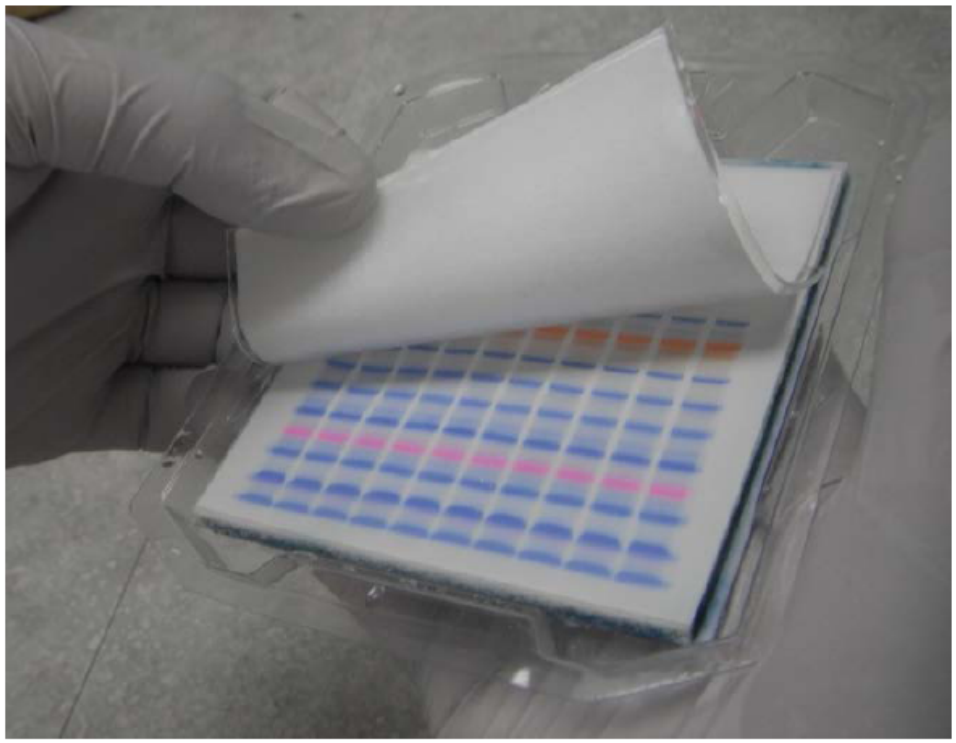
To obtain good transfer and detection results, open the device and disassemble the stack within 30 minutes of ending the blotting procedure.

1. Open the lid of the iBlot™ 2 Gel Transfer Device.

2. Discard the iBlot™ 2 Absorbent Pad and Top Stack.

3. Carefully remove and discard the gel and filter paper (if used) as shown below. Remove the transfer membrane from the stack and proceed with the blocking procedure or stain the membrane (see for details).

   Note: If you are using PVDF membranes, place the membrane immediately into water, as PVDF membranes dry quickly. If the PVDF membrane is dried, re-wet the membrane with methanol and rinse with deionized water a few times before use. Transfer the membrane to your blocking or staining solution only after you are sure that is completely wet, as reactivating after the membrane is exposed to the blocking solution may be problematic.



4. Remove and discard the plastic tray containing the Bottom stack.

5. Wipe down instrument and metal contacts with a damp cloth or paper tissue to remove any excess liquid that may have not been absorbed by the iBlot™ 2 Absorbent Pad.

6. At this point, the iBlot™ 2 Gel Transfer Device is ready for another run (no cooling period is required). If you are not using the device, turn off the power switch located on the back of the iBlot™ 2 Gel Transfer Device.

IMPORTANT! Do not reuse the iBlot™ 2 Absorbent Pad, iBlot™ Filter Paper, or Top and Bottom Stacks after blotting. Discard after each use.

3 Custom methods

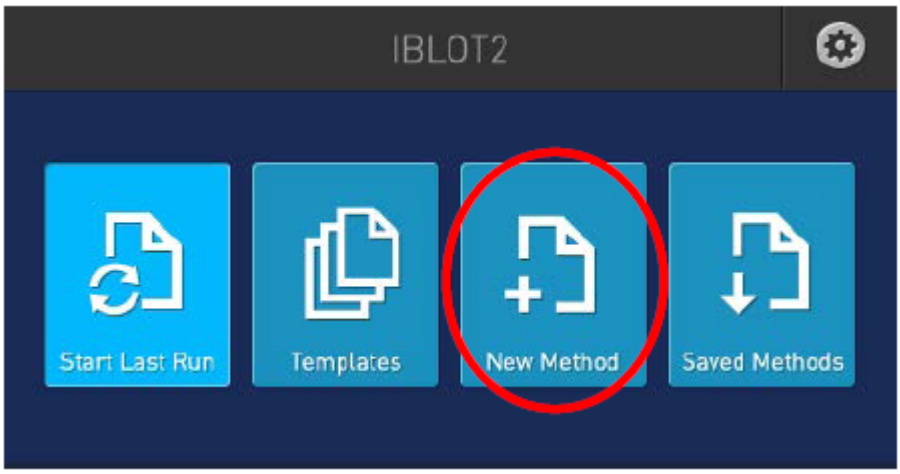
Creating custom methods

Introduction

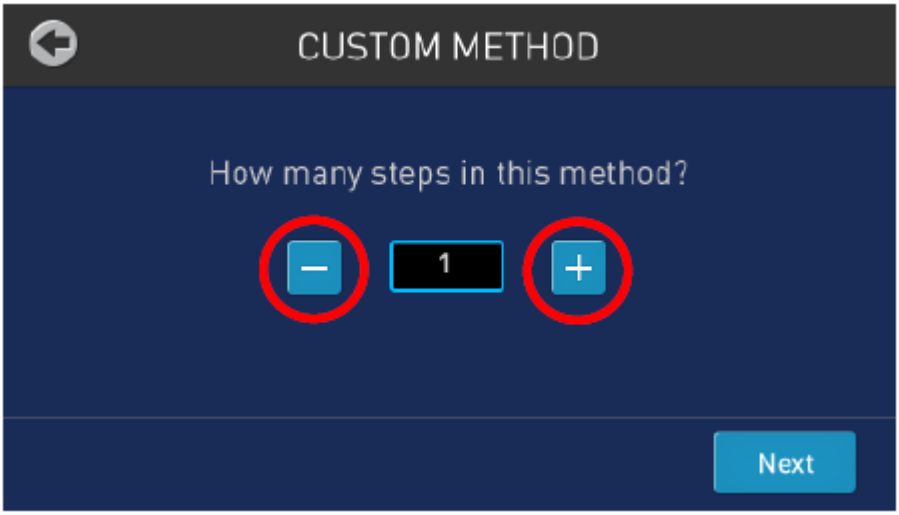
Custom methods can be created and saved on the iBlot™ 2 Gel Transfer Device for specific applications, or fine-tuning transfer conditions.

Programming a custom method

1. Select **New Method**.



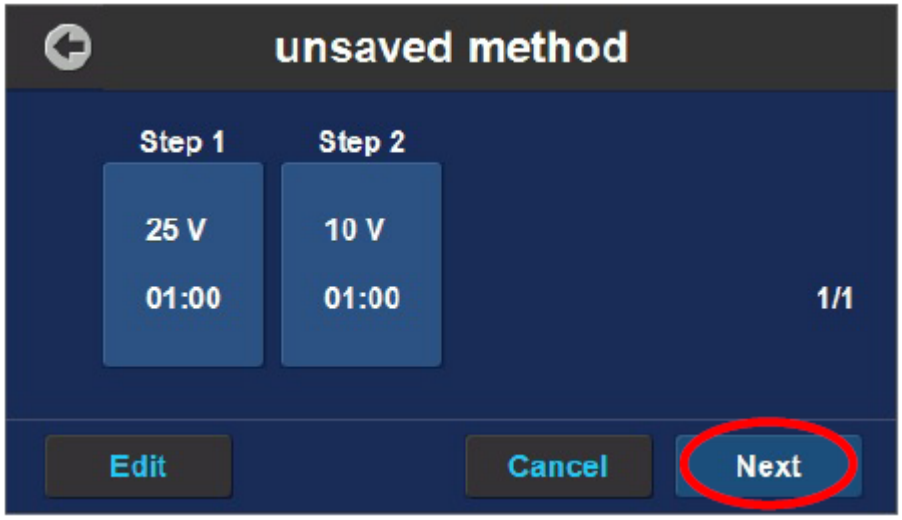
2. Select the number of steps to be included in the new Method using the (–/+) buttons. Up to 10 steps can be added to each Method.



3. Select the voltage and time (minutes and seconds) for each step by touching the desired field and using the keyboard to enter the appropriate value. Do not exceed the recommended time limit for voltage.

4. Select **Next** to go to the next step and repeat the procedure as necessary.

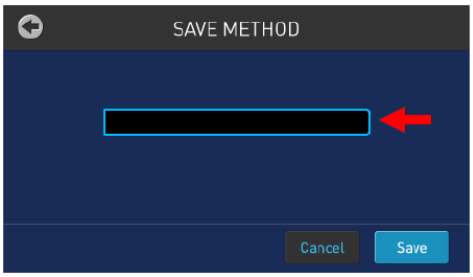
5. Select **Done** after programming the final step, and the entire Method is displayed. Select **Next** and proceed to to "Save a Custom Method" ("Save a custom method" on page 38).

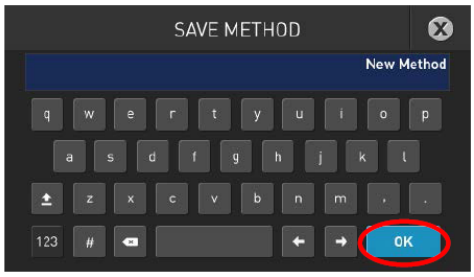


Note: The **Edit** button can be used at this point (see "Creating custom methods from a template" on page 39, "Creating Custom Methods from a Template"), but **do not** select **Cancel** without saving the Method or you will lose all of the information.

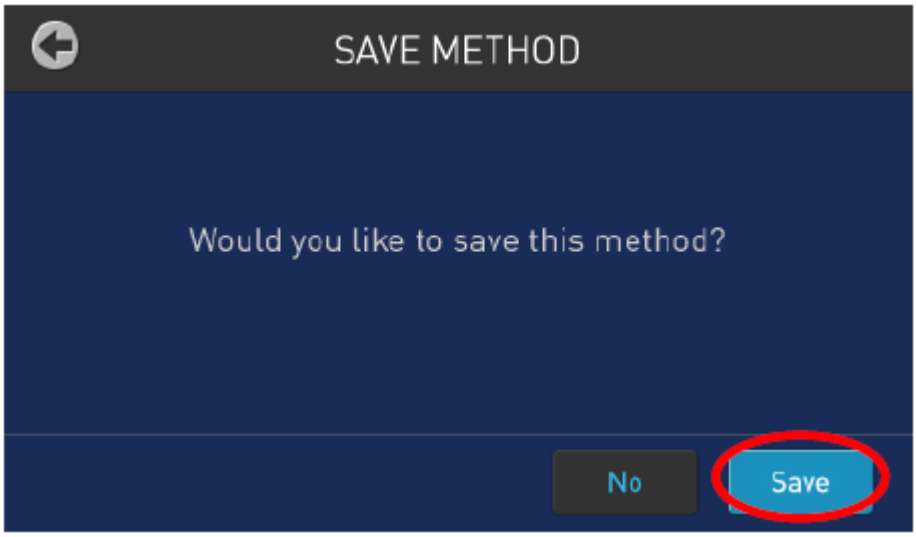
Save a custom method

1. Select the Name field, and use the keyboard to enter a name for the new Method.

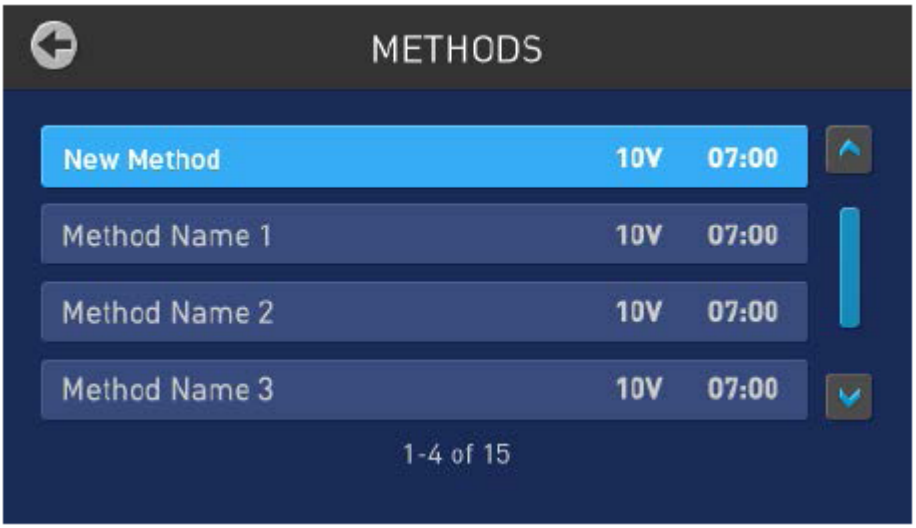




2. Save the method.



3. The new Method will appear in the list of Saved Methods.



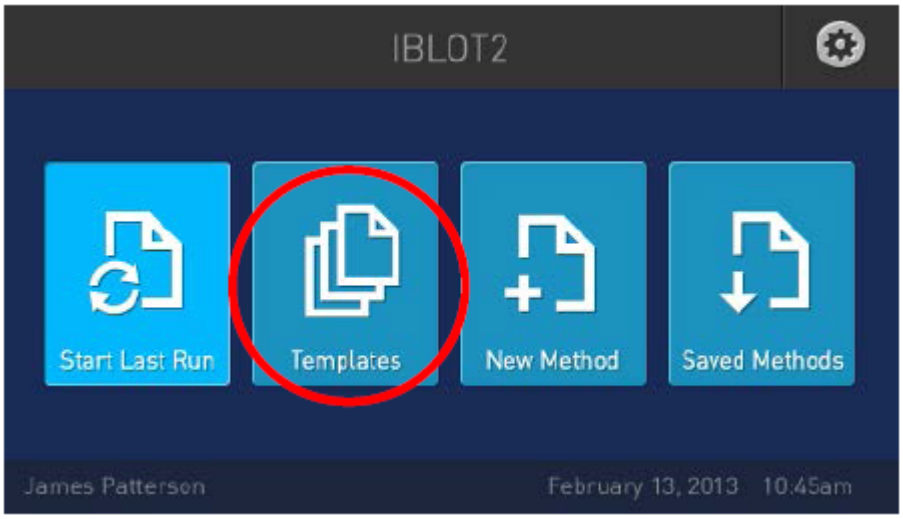
Creating custom methods from a template

Introduction

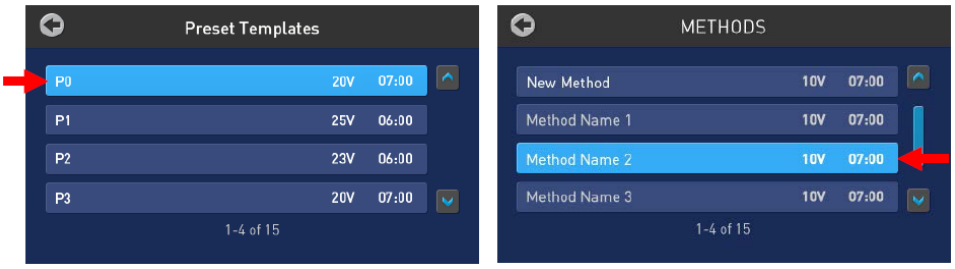
Custom methods can be created and saved on the iBlot™ 2 Gel Transfer Device for specific applications, or fine-tuning transfer conditions using a pre-set template or previously saved Method as a starting point.

Select a template

1. Select **Templates**.



2. Select a Method from the list on the Preset Templates or Saved Methods screen.

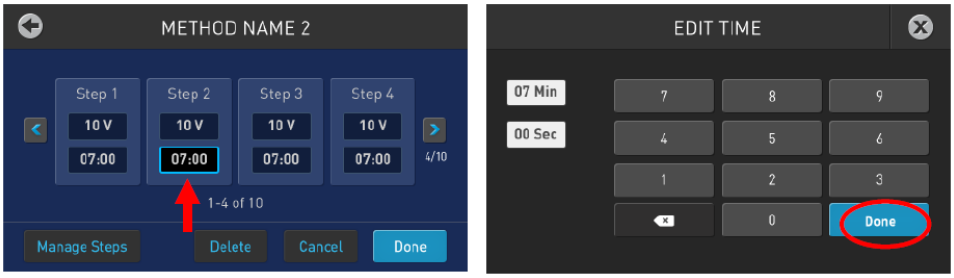


3. Select **Edit** from the Edit Method screen to enter Edit Mode (all voltage and time fields become enabled for editing).

4. Continue to "Change voltage/time" ("Change voltage/time" on page 40), or "Add/remove steps" ("Add/remove steps" on page 41).

Change voltage/time

1. Select voltage or time fields in any given step while in Edit Mode and a keyboard will appear.



2. Enter desired values for voltage or time and select **Done**.

3. After editing, the Edit Method screen is shown again.

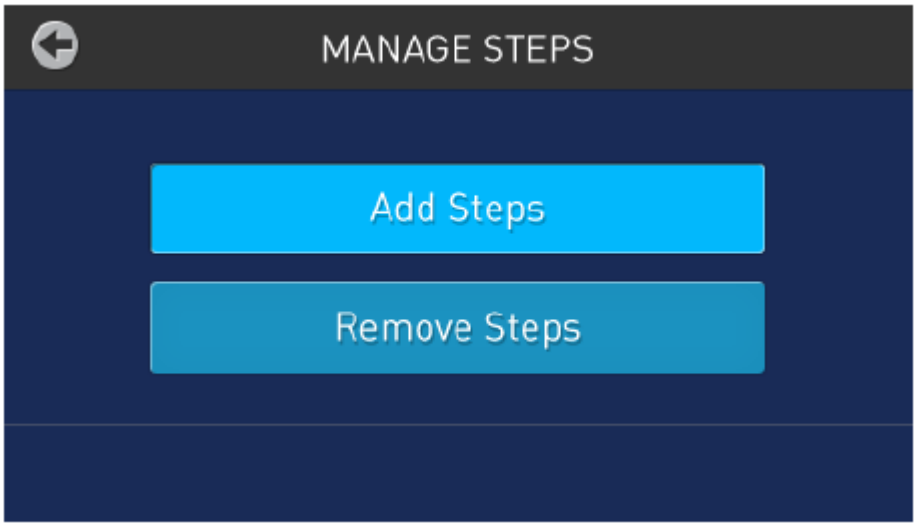
4. Select **Edit** to continue editing additional fields,
   OR

5. Select **Done** when editing has been completed and proceed to to "Save a Custom Method" ("Save a custom method" on page 43).

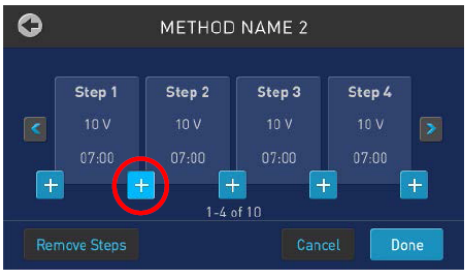
Add/remove steps

1. Select **Manage Steps** while in Edit Mode.

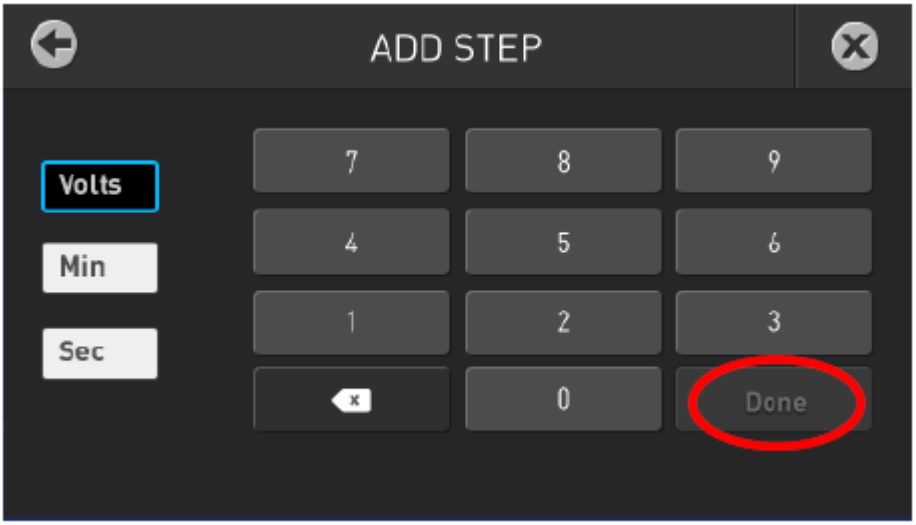
2. Select either **Add Steps** or **Remove Steps**.



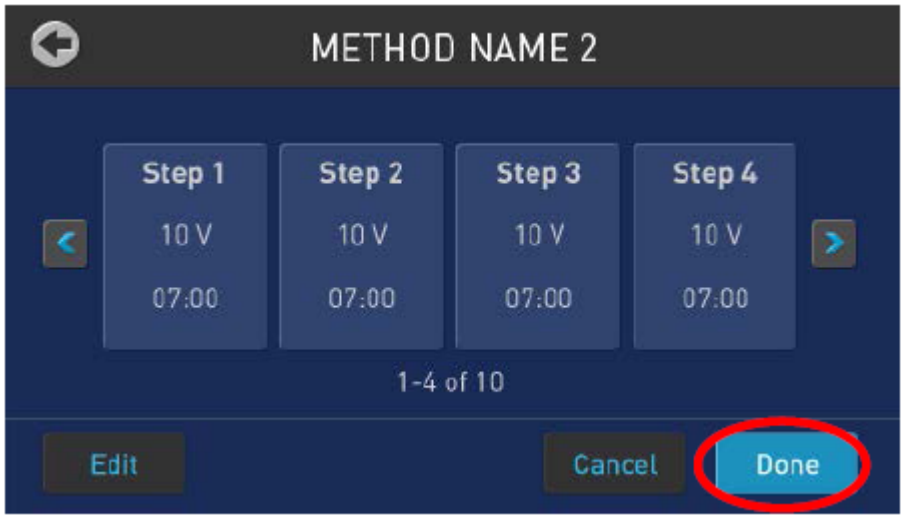
3. Select the + icon located at the location where you want to insert a step, or the – icon to remove a step.



4. If a step is added, a screen appears to enter the desired voltage and time for that step. After the values are added, select **Done** to return to the Edit Method screen.

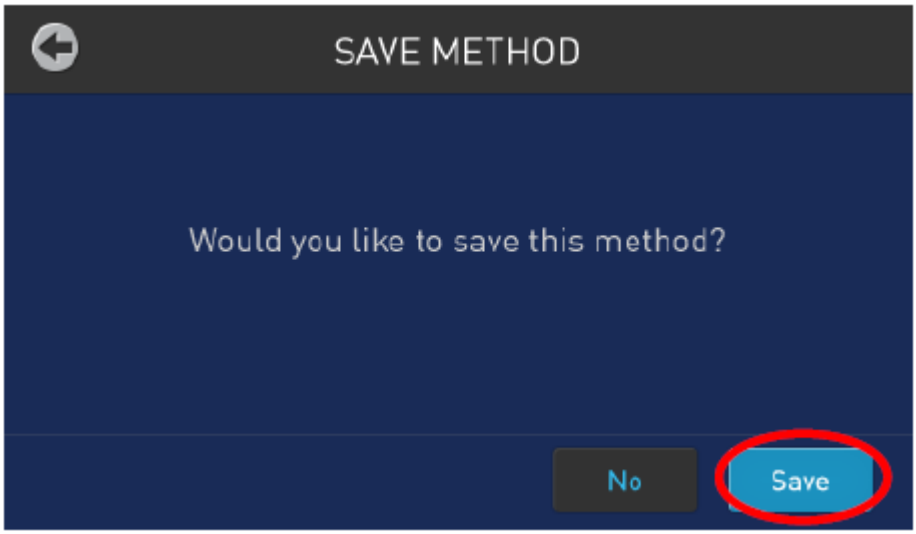


5. Select **Done** on the Edit Method screen and proceed to to "Save a Custom Method" ("Save a custom method" on page 43)

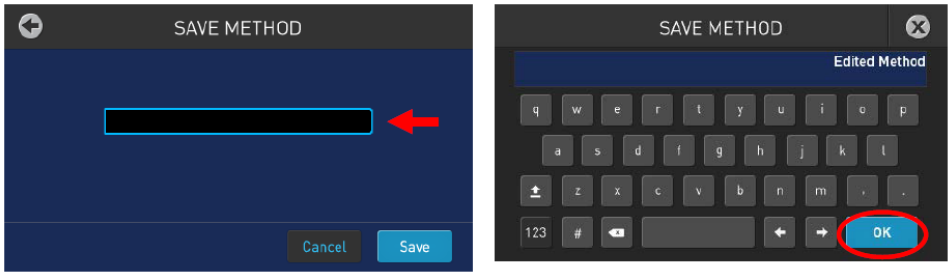


Save a custom method

1. Click **Save** to save the method.



2. Touch the name field on the Name Display screen and use the keyboard to enter in the characters. Touch **OK** when done.



3. The new Method will now appear under Saved Methods icon button.

A

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the iBlot™ 2 Gel Transfer Device and iBlot™ 2 Transfer Stacks.

To troubleshoot the immunodetection process, refer to the instructions supplied by the manufacturer of the immunodetection reagents.

Observation	Possible cause	Recommended action
No current.	Incorrect placement of the plastic tray leading to interference with contacts.	For **Mini** stacks (contact in center position), slide tray down slightly towards the front of the unit.
		For **Regular** stacks (contact on the side position) slide tray slightly towards the right.
	Incorrect placement of iBlot™ 2 Absorbent Pad contact.	Make sure the electrical contact of the iBlot™ 2 Absorbent Pad is aligned with the corresponding electrical contacts on the blotting surface of the iBlot™2 Gel Transfer Device.
	Plastic separator was not removed when assembling stack.	Make sure that the plastic separator is removed from the stack (see "Assembling the iBlot™ 2 Transfer Stack" on page 29).
	The metal safety contacts in the lid hinge may have been dirty and did not make contact.	Clean the metal safety contacts in the lid hinge with a cotton swab and water.Inspect metal safety contacts, contacts may need to be replaced ((Cat. No. IB28001)) as part of routine maintenance.
	One or more metal contacts were bent and unable to make good contact with the base unit.	Do not press forcibly on lid in an attempt to engage the contacts. Excessive force can cause the contacts to shift out of position and damage the unit. Contacts may need to be replaced ((Cat. No. IB28001)) as part of routine maintenance.
	Pressure plate malfunction.	Ensure the pressure plate in the lid pushes up and is not bound in any way.
	Top Stack was placed on the device upside-down.	Make sure the Top Stack is assembled with the copper electrode facing up.

Observation	Possible cause	Recommended action
Difficulty closing lid. **Note:** Do not press forcibly on the lid as it can cause damage to the unit.	Transfer stack was too thick.	The iBlot™ 2 Gel Transfer Device can only support gels of ≤1.5mm thickness. Ensure the proper gel is being used.
		Ensure that additional filter pads have not been added. Only use the supplied filter paper with the iBlot™ 2 Transfer Stacks.
		Ensure the stack tray is properly aligned. Do not remove the transfer stack from the plastic sample tray.
No proteins transferred to the membrane.	No current or incorrect method used.	See previous page to ensure the electrical circuit is complete and current is flowing through the device. Be sure to use the correct Method ("Recommended running parameters" on page 23).
Empty spots on the membrane.	Presence of air bubbles between the gel and the membrane prevented the transfer of proteins.	Be sure to remove all air bubbles between the gel and the membrane using the Blotting Roller.
	Expired or creased membranes were used.	Use the iBlot™ 2 Transfer Stacks before the expiration date printed on the package.
High molecular weight proteins remain in the gel indicated by staining of the gel after transfer.	Incorrect method or transfer conditions used. **Note:** It is normal for some proteins to remain in the gel because some high molecular weight proteins do not transfer completely using the iBlot™ 2 Gel Transfer Device, compared to semi-wet transfer apparatus.	Use the appropriate method and run time based on the gel type as described "Recommended running parameters" on page 23.
		For mini or midi gels: • Perform ethanol equilibration step as described "Optimizing blotting" on page 49 to improve transfer. • Use a Tris-acetate gel to separate the high molecular weight proteins. • Increase the transfer time in 30-second increments.
		For E-PAGE™ gels: • Increase the transfer time in 30-second increments. • Use Method P3 for 8 minutes.
Protein blow-through.	Transfer time was too long.	Reduce transfer time by 30-second increments. **Note:** Pre-stained markers are charged and tend to blow-through more than regular proteins.
Protein bands distorted on membrane.	Non-uniform electric field created around wells.	Ensure the gel is properly flattened using the Blotting Roller. Follow the recommendations on page 20 to obtain good results.

Observation	Possible cause	Recommended action
Protein not binding/transferring to membrane (PVDF).	PVDF membrane was dry/partially dry.	Regions where PVDF membranes are dry appear whiter than places where the membrane is wet. Remove the membrane, reactivate in 100% methanol, and rinse in water before reapplying to the transfer stack.
High background.	TBST buffers were used for washing.	Use PBST or WesternBreeze™ wash solutions.
Signal intensity is similar for different protein loads after detection.	High protein load (detection of is not within the linear range).	Since the immunodetection sensitivity is higher for dry blotting with the iBlot™ 2 Gel Transfer Device than for semi-dry or wet blotting, we recommend that you decrease the protein load, use more diluted antibody, or perform detection for shorter time. You may need to perform some optimization based on your initial results.
Corrosion of the Top Stack.	Incorrect placement of the Top Stack.	Be sure the Top Stack is placed correctly with the copper electrode facing up. Avoid placing the Top Stack in the inverted position.
Membrane and the gel turns blue.	Longer transfer times resulted in the deposition of copper ions.	Be sure to perform the transfer for the recommended time for each gel type.
Green discoloration of membrane edges.	Copper ions carried with liquids reached the membrane.	These deposits do not interfere with downstream processes. The stained regions can be cut away, but membrane washing typically results in their removal.
Bottom Stack transfer gel melts to a viscous blue solution.	Membrane was trimmed to fit the gel size, resulting in direct contact between the Top and Bottom Stacks.	Always maintain the membrane size identical to the transfer stack. Transfer quality is not affected by smaller gel size compared to the membrane.

B

Post transfer analysis

Post transfer analysis

After the transfer, proceed to immunodetection, store the membrane for future use, or stain the membrane.

- For immunodetection of proteins, use the WesternBreeze™ Chromogenic or Chemiluminescent Immunodetection Kits available at **thermofisher.com** (see "iBlot™ 2 Transfer Stacks" on page 57–"Additional products" on page 57), or any other immunodetection kit.

  Note: When using the iBlot™ 2 Dry Blotting System to transfer proteins from SDS-PAGE gels, the applied field strength can result in the partial depletion of negative ions bound to the proteins. This may result in a slight decrease in the amount of protein migrating from the gel, but it also results in improved binding of the transferred proteins to the membrane. Since the membrane maintains the protein load better, higher sensitivity can be achieved for subsequent immunodetection procedures.

- To store nitrocellulose membranes, air-dry the membrane and store the membrane in an air-tight plastic bag at room temperature or 4°C. Avoid storing nitrocellulose at temperatures below –20°C. Low temperatures cause the nitrocellulose to turn brittle.
- To store PVDF membranes, air-dry the membrane and store the membrane in an air-tight plastic bag at room temperature, 4°C, or –80°C. When you are ready to use the membrane, re-wet the membrane with methanol for a few seconds, then rinse the membrane thoroughly with deionized water to remove methanol.
- To stain membranes after blotting, use any method of staining for total protein visualization, such as Coomassie™ Blue R-250, Ponceau S, Amido Black, Novex™ Reversible Membrane Protein Stain Kit, or SYPRO™ Ruby™ Blot Stain ("Additional products" on page 57). The iBlot™ 2 Gel Transfer Device blotting protocol is compatible with most of the staining methods listed above.

  Note: The sensitivity of total protein membrane staining after blotting with the iBlot™ 2 Gel Transfer Device is slightly lower than the total membrane protein staining obtained with the semi-wet transfer protocol. However, due to the nature of dry blotting, lower transfer does not affect the immunodetection sensitivity.

If you do not detect any proteins on the membrane after immunodetection or staining, refer to Troubleshooting "Introduction" on page 45. Refer to the manufacturer recommendations for optimizing immunodetection.

The immunodetection profile of proteins transferred using the iBlot™ 2 Dry Blotting System may differ from what is observed when using other transfer methods, such as traditional semi-dry or wet blotting systems. It is recommended to optimize parameters such as gel protein load, primary and secondary antibody dilution, and exposure time (see "Optimizing blotting" on page 49 for details) when using the iBlot™ 2 Dry Blotting System for the first time with any new combination of antigen and detection reagents.

C

Optimizing blotting

Optimizing blotting

When using the iBlot™ 2 Gel Transfer Device, most proteins transfer efficiently using the protocol in this manual. Based on specific properties of a protein or a set of proteins, some optimization of the blotting protocol may be necessary.

Optimize blotting as follows:

Perform an ethanol equilibration step prior to transfer

To improve the transfer of high-molecular weight proteins from mini- or midi-NuPAGE™ or Tris-Glycine gels, submerge the gel in 20% ethanol (prepared in deionized water), and equilibrate for 5–10 minutes at room temperature on a shaker prior to transfer.

Do not equilibrate for longer than 10 minutes, or sensitivity may be reduced. After equilibration, perform transfer using the iBlot™ 2 Gel Transfer Device as described in this manual.

Increase or decrease transfer time

Based on the initial results, you can increase or decrease the transfer time for the Method used to perform the transfer (refer to Chapter 3, "Custom methods" for details on customizing a Method).

Do not perform transfer for more than the recommended run time limit indicated for each Method ("Description of methods" on page 23).

Proteins >150 kDa migrate more slowly, and require more time to transfer. If your protein of interest is in this size range, it may be necessary to use a Run Time of 8–10 minutes for your transfer.

Small proteins <30 kDa migrate more rapidly during electrophoretic separation, and consequently require less time to transfer from the gel matrix to the membrane. If your protein of interest is in this size range, you may need to reduce the Run Time to 5–6 minutes for your transfer.

It is normal for some proteins to remain in the gel, because some high molecular weight proteins do not transfer completely using the iBlot™ 2 Gel Transfer Device as compared to wet transfer apparatus.

Since the sensitivity of detection using the iBlot™ 2 Gel Transfer Device is higher than that of semi-wet and semi-dry blotting, complete transfer of proteins is not required.

Near-complete transfer of prestained standard protein bands is observed with the iBlot™ 2 Gel Transfer Device. However, note that the complete transfer of prestained protein standards does not indicate complete transfer of other proteins or blow-through of other proteins.

D

Maintenance

Cleaning

Before cleaning the iBlot™ 2 Gel Transfer Device, make sure the device is turned off.

Wipe off the blotting surface and electrodes with a damp cloth or paper tissue.

Allow the parts to dry before use.

General maintenance

To avoid damaging the iBlot™ 2 Gel Transfer Device, do not perform any repairs or service other than general maintenance on the iBlot™ 2 Gel Transfer Device.

Instructions are provided below for replacing fuses and electrical contacts.

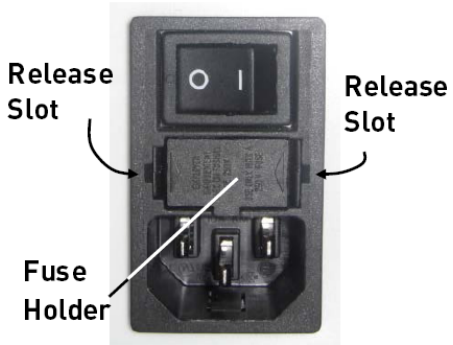
Disconnect the unit from the power supply by removing the power cord before performing either of these maintenance procedures.

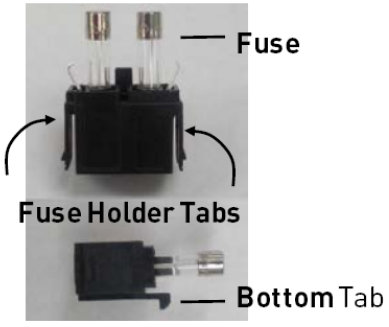
For any other repairs and service, contact Technical Support ().

Replacing the fuse

The fuses for the device are located at the rear of the device. To replace a fuse:

- Make sure the device power is OFF.
- Disengage the fuse holder by depressing the tabs via the release slots.
- Remove the fuse holder and change the fuse (see "iBlot™ 2 Gel Transfer Device specifications" on page 55 for fuse specifications).
- Replace the fuse holder (with the bottom tab down).



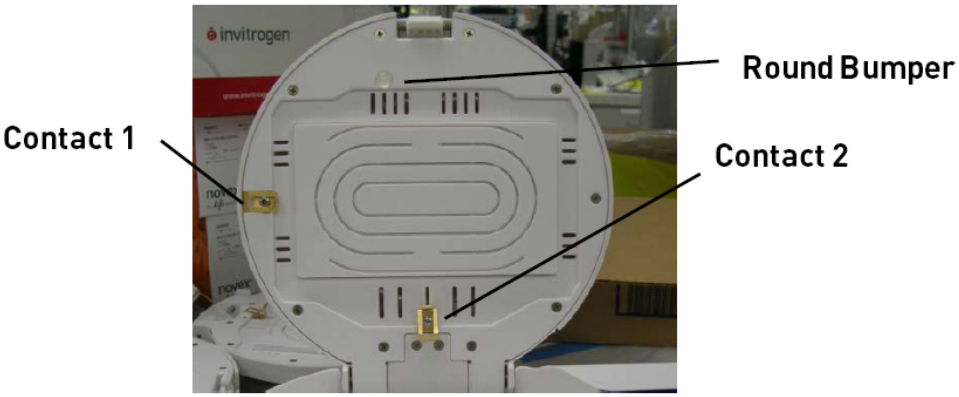


Replacing electrical contacts

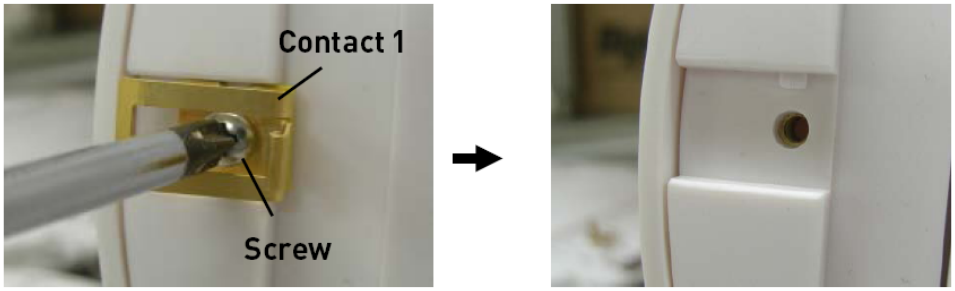
Electrical contacts should be inspected and replaced regularly as part of routine maintenance. Overtime, electrical contacts can become depressed and worn out. To ensure optimal life of the electrical contacts, wipe down the instrument and contacts with a damp cloth after each use.

To replace an electrical contact, a #1 Phillips Screwdriver is required.

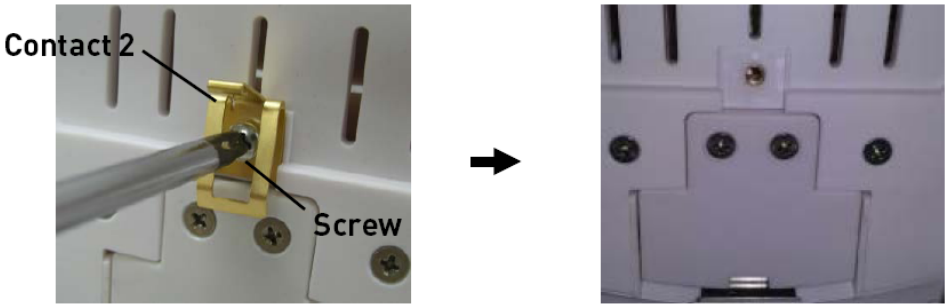
1. Make sure the device power is OFF, then open the lid of the iBlot™ 2 Gel Transfer Device.



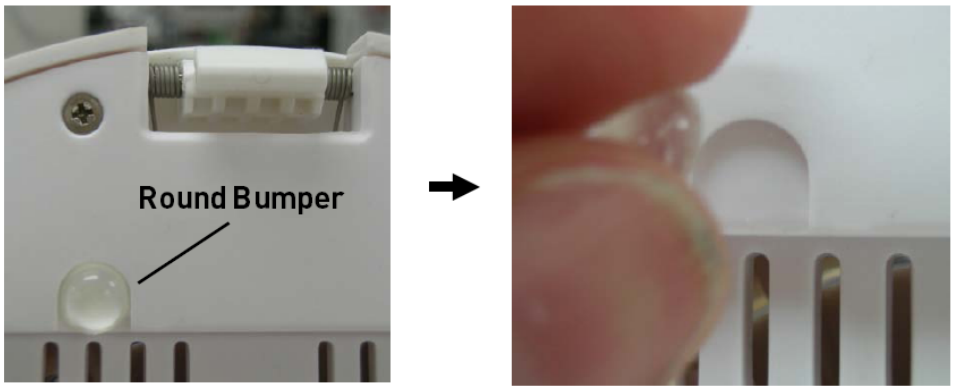
2. Hold the Lid with one hand, and remove the screw holding Contact 1. Discard both the screw and the contact.



3. Remove the screw holding Contact 2. Discard both the screw and the contact.

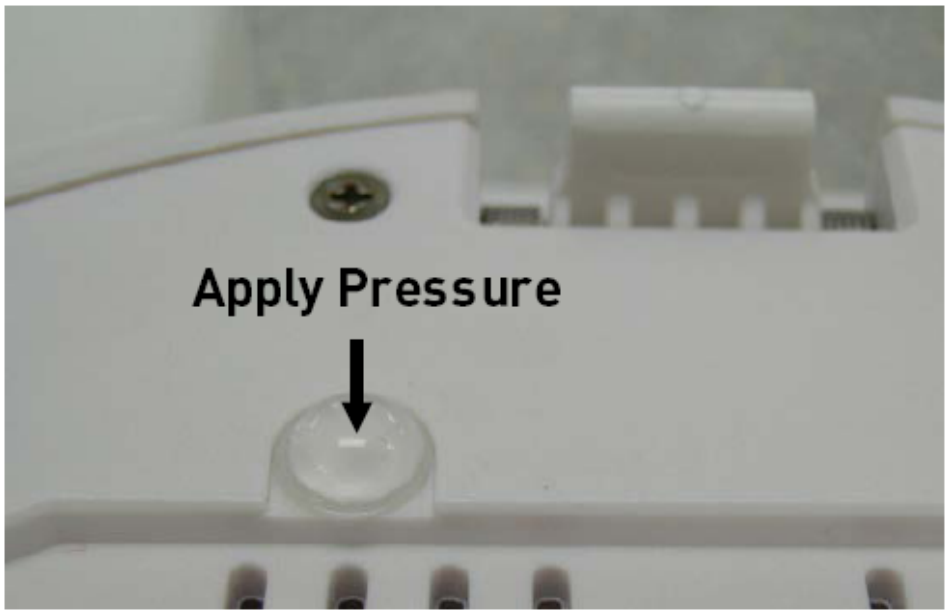


4. Hold the Lid with one hand, and unpeel the Round Bumper from the Lid. Discard the Round Bumper.

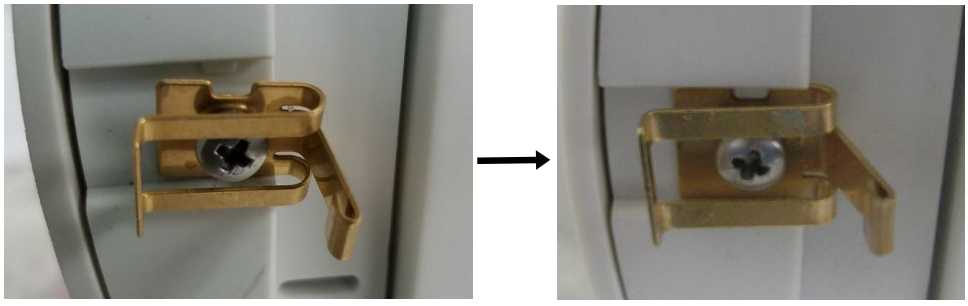


5. Open the bag of the Electrode Replacement Kit (Cat. no. IB28001), and remove all of the parts.

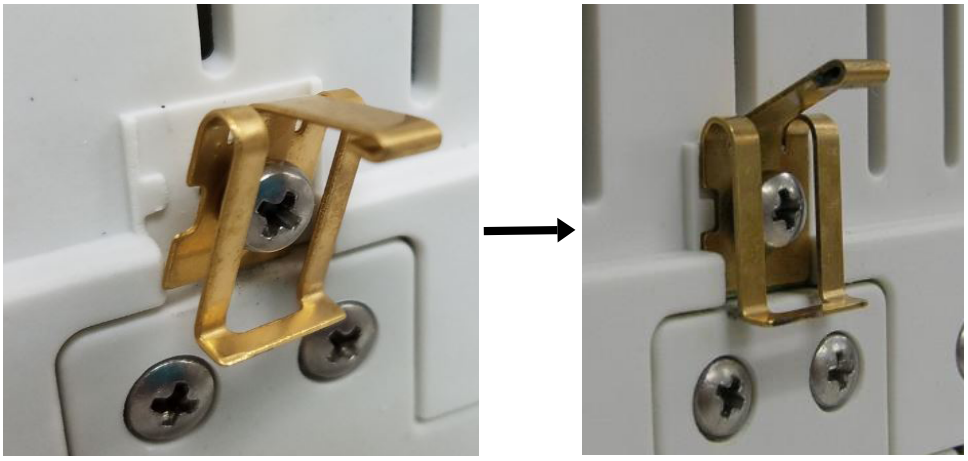
6. Peel off the backing from the Round Bumper and place the bumper into the recess. Hold the Lid with one hand and press down on the bumper for five seconds to ensure proper adhesion.



7. Place a screw through the hole in one of the contacts. Position the contact/screw onto the Contact 1 location. Note: the contact has a small orientation notch which matches a bump in the Lid. This is to ensure proper orientation of the contact when assembled. Turn the screw with a screwdriver until it is hand-tight.



8. Place a screw through the hole in one of the contacts. Position the contact/screw onto the Contact 2 location. Turn the screw with a screwdriver until it is hand-tight.



E Product specifications

iBlot™ 2 Gel Transfer Device specifications

Dimensions:	37 cm (l) × 20 cm (w) × 11 cm (h)
Weight:	2.3 kg
Electrical Parameters:	100–240 V, 50/60 Hz, 6.3 A
Built-in Features:	Digital display, alarm
Compatibility:	Suitable for transfer of mini- (8 × 8 cm), midi- (8 × 13 cm), and E-PAGE™ Gels
iBlot™ 2 Materials:	Polycarbonate, Cycoloy, Acrylic, Gold plated copper, Stainless steel, Plasticized silicone, Aluminum
Operating Temperature:	5–40℃
Blotting Roller:	Delrin™ roller (8.6-cm wide) attached to a stainless steel handle
Replacement Fuse:	6.3A Slo-Blo, 5x20, 250V (Littelfuse 021806.3HXP)

The iBlot™ 2 Gel Transfer Device is impervious to alcohol, acid (HCl), alkali (NaOH) but not compatible with acetone, dimethyl sulfoxide, and acetic acid.

iBlot™ 2 Transfer Stack specifications

Specifications for the iBlot™ 2 Transfer Stacks are listed below. For a more detailed description of the iBlot™ 2 Transfer Stacks, see "iBlot™ 2 Transfer Stacks" on page 15.

Top Stack	
Regular Top Stack Gel Layer:	13.6 cm (l) × 8.5 cm (w) × 0.19 cm (thick)
Mini Top Stack Gel Layer:	8.5 cm (l) × 8.5 cm (w) × 0.19 cm (thick)
Top Stack Gel Layer Composition:	Proprietary
Electrode:	Copper-coated mesh
Bottom Stack	
Regular Bottom Stack Gel Layer:	14.1 cm (l) × 8.5 cm (w) × 0.32 cm (thick)

Top Stack	
Mini Bottom Stack Gel Layer:	8.5 cm (l) × 8.5 cm (w) × 0.32 cm (thick)
Bottom Stack Gel Layer Composition:	Proprietary
Electrode:	Copper-coated mesh
Transfer Membrane:	Nitrocellulose (0.2 µm) or PVDF (0.2 µm, low fluorescence)
Plastic Tray:	16.8 cm × 10.3 cm (1.7-cm wide copper contact)
iBlot™ 2 Absorbent Pad	
Dimensions:	15 cm (l) × 9.5 cm (w) × 1.1 cm (thick)
Material:	Gray Melamine
Metal Contact:	Aluminum
iBlot™ Filter Paper	
Regular Filter Paper:	13.5 cm (l) × 8 cm (l) × 0.04 cm (thick)
Mini Filter Paper:	8 cm (l) × 8 cm (w) × 0.04 cm (thick)

F

Accessory products

iBlot™ 2 Transfer Stacks

iBlot™ 2 Transfer Stacks are available at **thermofisher.com**. Ordering information is provided below.

Product	Quantity	Cat. No.
iBlot™ 2 Regular Transfer Stack, Nitrocellulose	1 pack of 10	IB23001
iBlot™ 2 Regular Transfer Stack, PVDF	1 pack of 10	IB24001
iBlot™ 2 Mini Transfer Stack, Nitrocellulose	1 pack of 10	IB23002
iBlot™ 2 Mini Transfer Stack, PVDF	1 pack of 10	IB24002
Blotting Roller	1 unit	LC2100

Additional products

Additional reagents that may be used for electrophoresis of proteins are available at **thermofisher.com**. Ordering information is provided below. For more information, visit **thermofisher.com** or call Technical Support ().

Product	Quantity	Cat. No.
iBind™ Flex Western Device	1 Device	SLF2000
iBind™ Flex Cards	10 Cards	SFL2010
iBind™ Flex Solution Kit	1 Kit	SLF2020
SuperBlock™ (PBS) Blocking Buffer	1 L	37515
SuperBlock™ T20 (PBS) Blocking Buffer	1 L	37516
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	200 mL	34580

Product	Quantity	Cat. No.
SuperSignal™ West Dura Extended Duration Substrate	100 mL	34075
SuperSignal™ West Femto Maximum Sensitivity Substrate	100 mL	34095
SeeBlue™ PLUS™2 Pre-Stained Standard	500 µL	LC5925
MagicMark™ XP Western Protein Standard	250 µL	LC5602
Novex™ Reversible Membrane Protein Stain Kit	1 kit	IB7710
SYPRO™ Ruby™ Protein Blot Stain	200 mL	S-11791

Precast gels and premade buffers

A large variety of precast gels including Bolt™, NuPAGE™ Novex™, Tris-Glycine mini- and midi gels, and E-PAGE™ gels, as well as premade buffers are available at **thermofisher.com**.

G

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

AVERTISSEMENT ! Installation des fusibles. Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Symbols on instrument

The symbols used on the iBlot™ 2 Gel Transfer Device are explained below:

Symbol	Information
CE	The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required.
	The iBlot™ 2 Gel Transfer Device complies with the TUV Rhineland North America Inc. safety requirements. The indicators "C" and "US" means that the product is certified for both the U.S. and Canadian markets, to the applicable U.S. and Canadian standards.
	The Caution symbol denotes a risk of safety hazard. Refer to accompanying documentation to avoid possible personal injury or instrument damage.
	The WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE. Visit http://www.lifetechnologies.com/weee for collection and recycling options.
	The Electrical Warning symbol denotes a risk of electrical shock hazards. To avoid risk of injury from electric shock, do not open enclosures marked with this symbol.
	The Caution symbol denotes a risk of safety hazard. Refer to accompanying documentation to avoid possible personal injury or instrument damage.
REF	Product catalog number.
	Consult instructions for use.
	Site of manufacture.

Safety standards

Reference	Description
EN-61010-1:2010 (3rd Edition)	*Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 1: General Requirements*

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"

Reference	Description
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices*

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive"—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union "RoHS Directive"—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	"China RoHS" Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).

- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

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    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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