invitrogen

iBlot™ 3 Western Blot Transfer System

USER GUIDE

For dry electroblotting of proteins from mini and midi gels

Catalog Number IB31001

Publication Number MAN0028464

Revision C

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.

Revision history: MAN0028464 C (English)

Revision	Date	Description
C	12 March 2025	Add information about iBlot™ 3 Low Flurorescence PVDF Transfer Stacks.
B	16 August 2024	Adding edits to various sections for clarification.
A.0	9 January 2023	New document for iBlot™ 3 Western Blot Transfer System.

The information in this guide is subject to change without notice.

Contents

1 Product information

Product contents

iBlot™ 3 Western Blot Transfer Device contents

This manual is intended to provide instructions for the operation of the iBlot™ 3 Western Blot Transfer Device (Cat. No.IB31001).

The contents of the iBlot™ 3 Western Blot Transfer Device are listed below. See “iBlot™ 3 Western Blot Transfer Device specifications” on page 42 for specifications and description of the iBlot™ 3 Western Blot Transfer Device.

Component	Quantity
iBlot™ 3 Western Blot Transfer Device	1
Blotting Roller	1
Power Cord	1

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. Additional safety information is available in the safety Appendix G on page 46.

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™ 3 Western Blot 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™ 3 Western Blot Transfer Device is subject to the conditions described in this manual.

Operation of the iBlot™ 3 Western Blot Transfer Device is subject to the following conditions:

- Indoor use
- Altitude below 2,000 meters
- Temperature range: 4–30°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™ 3 Western Blot Transfer Device. To honor the warranty, the iBlot™ 3 Western Blot 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™ 3 Western Blot Transfer Device

- Remove the roller from the Styrofoam packaging.
- Remove the device from the Styrofoam packaging.
- Remove the protective film from the touch screen.
- Remove blue protective tape from the electrode contact positions on the base.

iBlot™ 3 Western Blot Transfer Device

Device views

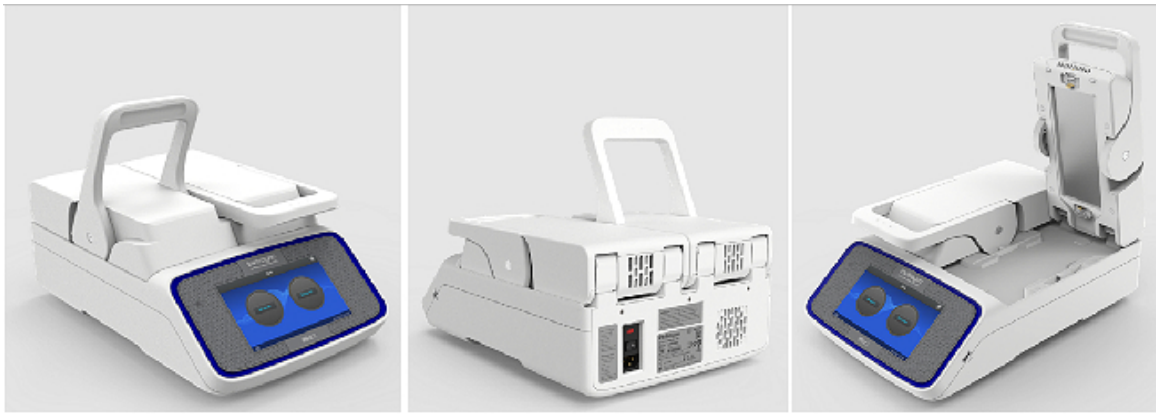


Figure 1 Front, rear, and top views of the iBlot™ 3 Western Blot Transfer Device

About the system

iBlot™ 3 Western Blot Transfer System

The iBlot™ 3 Western Blot Transfer System consists of the iBlot™ 3 Western Blot Transfer Device and associated iBlot™ 3 Transfer Stacks (sold separately). The iBlot™ 3 Western Blot Transfer Device has a unique design, which, in conjunction with the patented gel matrix technology of the iBlot™ 3 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 can be performed with iBlot™ 3 Transfer Stacks in less than 10 minutes.

Features

- Pre-programmed methods for protein transfers from various gel types in 3–8 minutes
- Two independently controlled transfer stations allow one or more users to simultaneously run two different transfer methods
- Built-in cooling enables the user to have more control over the transfer temperature
- Enhanced user safety with built-in safety features in the device
- User-friendly design with an integrated power supply to avoid inconsistencies associated with the use of an external power supply
- Fast, reliable protein transfer with ready-to-use iBlot™ 3 Transfer Stacks with no need to prepare buffers or membranes
- iBlot™ 3 Transfer Stacks have reduced fluorescent background and copper greening compared to iBlot™ 2 Transfer Stacks
- iBlot™ 3 Low Fluorescence PVDF Stacks have membranes packaged separately (not part of the stack) for optimal low autofluroscence and low background
- Compatible for use with Novex™ and NuPAGE™ gel types and other gels
- Not compatible with E-PAGE™ gels

System components

iBlot™ 3 Western Blot Transfer Device

The iBlot™ 3 Western Blot Transfer Device is a self-contained blotting unit with integrated power supply used for fast dry blotting of proteins.

iBlot™ 3 Transfer Stacks

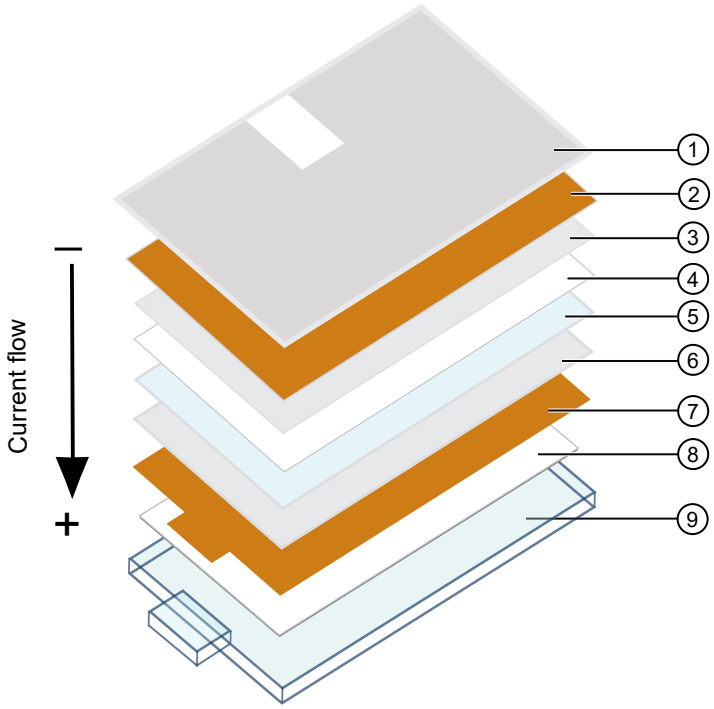
The iBlot™ 3 Transfer Stacks are disposable stacks that have integrated PVDF or nitrocellulose transfer membranes to perform dry blotting of proteins. Each iBlot™ 3 Transfer Stack contains a copper electrode and appropriate cathode and anode buffers in the gel matrix to allow fast, reliable transfer of proteins. See “iBlot™ 3 Transfer Stacks” on page 44 for details.

System overview

The iBlot™ 3 Western Blot Transfer System is based on the dry blotting concept, utilizing the unique, patented gel matrix technology developed for E-Gel™ gels and applied in iBlot™ 3 Transfer Stacks.

Each iBlot™ 3 Transfer Stack consists of a Bottom Stack and a Top Stack sandwiching a pre-run gel and a nitrocellulose or PVDF membrane. The iBlot™ 3 Transfer Stacks, Low Fluorescent PVDF do not include a membrane in the pre-assembled stacks. Instead, a pouch of 10 Low Fluorescence PVDF membranes is included in the box. The iBlot™ 3 Transfer Stacks are assembled with the blotting membrane on the anode side, and a pre-run gel on the cathode side.

Schematic of iBlot™ 3 Transfer Stack showing the flow of current



1. Top felt with folded aluminum tab
2. Copper cathode
3. Cathode gel matrix
4. Plastic divider
5. Membrane (NC or PVDF)
6. Anode gel matrix
7. Copper anode
8. Bottom felt
9. Transfer tray

After the stack is assembled on the iBlot™ 3 Western Blot Transfer Device, and the appropriate method is selected, the run is initiated. Transfer of proteins from the gel to the blotting membrane is accomplished in approximately 3–8 minutes. The rapid transfer without the need for external power

supply or premade buffers is possible due to the following features of the iBlot™ 3 Western Blot Transfer System:

- The gel matrix of the Bottom Stack and Top Stack incorporates the appropriate anode and cathode buffers to act as ion reservoirs. This format eliminates the need for premade buffers or soaked membranes 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 transfer stack gel matrix technology and reduced distance between the electrodes combined with the integrated power supply allows the system to generate high field strength and increase transfer speed.

Description of parts

iBlot™ 3 Western Blot Transfer Device

The iBlot™ 3 Western Blot 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 35 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. Pre-installed firmware controls the parameters such as voltage, time, and cooling, and allows selection of Methods (see “Description of methods” on page 20 for details on each Method).

IMPORTANT! When installing the iBlot™ 3 Western Blot 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 (“Device views” on page 8) are easily accessible.

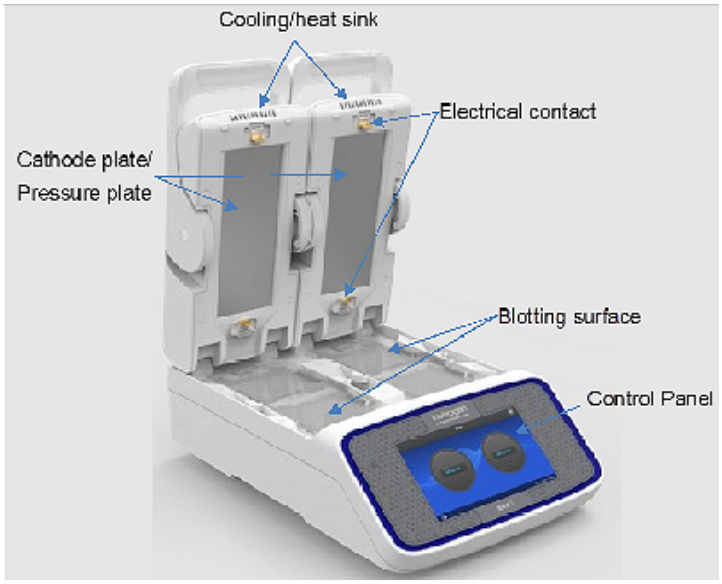


Figure 2 Device with lid open and detailed parts

Blotting surface

The blotting surface is the area where the iBlot™ 3 Transfer Stack containing the gels is placed to perform blotting. Alignment guides are used for proper orientation of midi and mini transfer stacks. Each station (Station 1 and Station 2) can accommodate one or two mini stacks, or one midi stack.

Lids and cooling

The lids of the iBlot™ 3 Western Blot Transfer Device exert even pressure on the stack surface when the lid is closed.

The lids contain a cooling system in the form of a heat sink connected to, and in thermal communication, with the cathode plate. During the transfer run, there is a heat exchange between the transfer stack and the cathode plate, and from the cathode plate back to the heat sink. The air surrounding the fins of the heat sink facilitates continuous cooling of the stack and the extent of cooling is controlled by the fan.

Control panel

The graphical Touch Screen User Interface is an LCD display allowing the user to select methods and control the device by following menu prompts.

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.

Power cord and power adapters

The Power Cord connects to the iBlot™ 3 Western Blot 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 (see “Replacing the fuse” on page 38 for a detail view of the switch) 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.

Use the appropriate power cord and power adapter for your geographical region.

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iBlot™ 3 Transfer Stacks

The iBlot™ 3 Transfer Stacks are used to transfer proteins from gels onto nitrocellulose, PVDF, and Low Fluorescence PVDF membranes, and are available in Midi size for blotting one midi or two mini gels, and Mini size for blotting one mini gel.

Guidelines for iBlot™ 3 Transfer Stacks

- Store the iBlot™ 3 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 free liquid, allowing for easier clean-up.
- **Discard the iBlot™ 3 Transfer Stack after every use. Do not reuse the iBlot™ 3 Transfer Stack or any of its components.**
- **iBlot™ 3 Transfer Stacks are compatible only with the iBlot™ 3 Western Blot Transfer Device.** Use iBlot™ 3 Transfer Stacks only for their designated application.

Note: The maximum voltage and current of the output to the gel stacks is 35 VDC and 6.5 A.

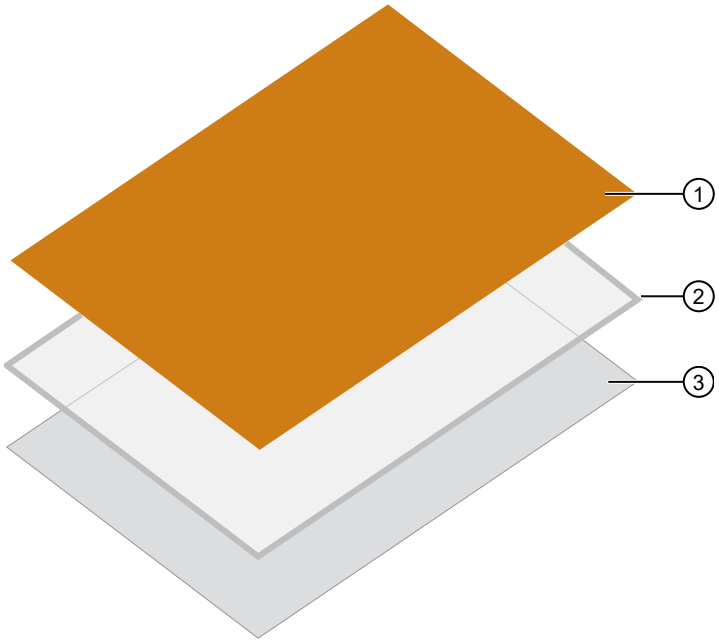
The following iBlot™ 3 Transfer Stacks are available at **thermofisher.com/iblot3** (see "iBlot™ 3 Transfer Stacks" on page 44 for ordering information).

Each iBlot™ 3 Transfer Stacks box comes with the following components: 10 iBlot™ 3 Transfer Stacks (mini or midi), 10 iBlot™ 3 Absorbent Pads (mini or midi), and 10 iBlot™ Filter Papers (mini or midi). The iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF come with the additional component, 10 iBlot™ 3 Low Fluorescence PVDF Membranes.

Product	Transfer Membrane	Cat. No.
iBlot™ 3 Transfer Stacks, Midi	Nitrocellulose	IB33001
	PVDF	IB34001
	Low Fluorescence PVDF	IB34003
iBlot™ 3 Transfer Stacks, Mini	Nitrocellulose	IB33002
	PVDF	IB34002
	Low Fluorescence PVDF	IB34004

Top stack

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



① Copper electrode
② Cathode gel matrix
③ Plastic divider

Bottom stack

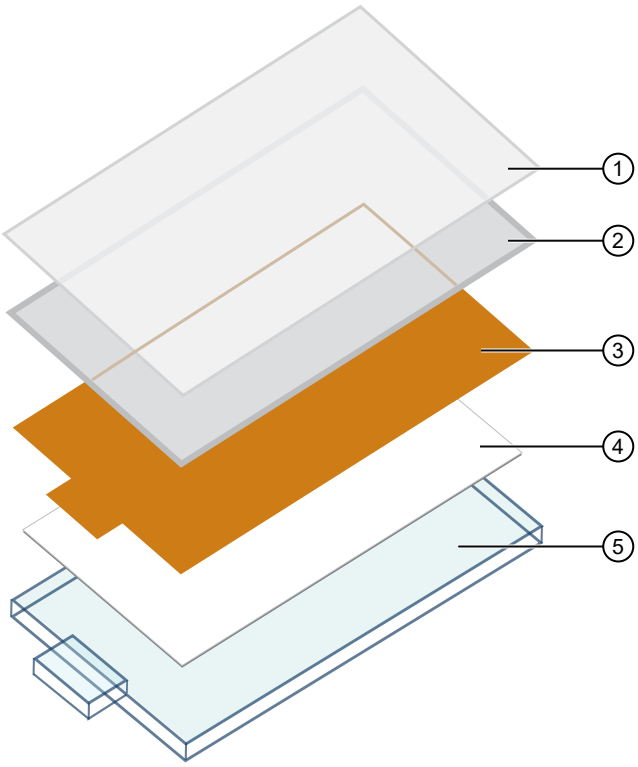
The Bottom Stack in the plastic tray contains an absorbent pad, a copper electrode, transfer anode gel layer, and a nitrocellulose or PVDF membrane for protein transfer.

Note: Low Fluorescence PVDF membrane is not part of the pre-assembled stack. Low Fluorescence PVDF is packaged separately in a pouch and requires activation in methanol or ethanol before use.

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™ 3 Transfer Stack is packaged serves as the support for assembling the transfer stack with the gel, and as a reservoir to contain any liquid generated during the blotting process.

The nitrocellulose and PVDF membranes **do not** require any pretreatment before use. However, the Low Fluorescence PVDF does require pre-treatment in methanol or ethanol before use. For more information, see “Assemble the iBlot™ 3 Transfer Stack” on page 24.

During the transfer, always use the Bottom Stack with the tray in the iBlot™ 3 Western Blot Transfer Device.



1. Blotting membrane
2. Anode gel matrix
3. Copper electrode
4. Absorbent pad
5. Plastic tray

Transfer membrane

The iBlot™ 3 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)**

  The PVDF membrane has higher binding capacity than nitrocellulose. **The PVDF membrane is preactivated and ready for use (no need for additional 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.

- **Low Fluorescence PVDF membrane (0.3 µm)**

  The Low Fluorescence PVDF membrane is packaged separately to ensure membrane integrity and low background. **The low-fluorescence membrane requires pre-activation in 100% methanol or 100% ethanol followed by a rinse in deionized water.** The membrane is hydrophobic, so it requires more volume to prevent the membrane from floating. The membrane is compatible with commonly used detection methods such as staining, immunodetection, fluorescence, and radiolabeling. The proteins bind to the membrane due to hydrophobic interactions.

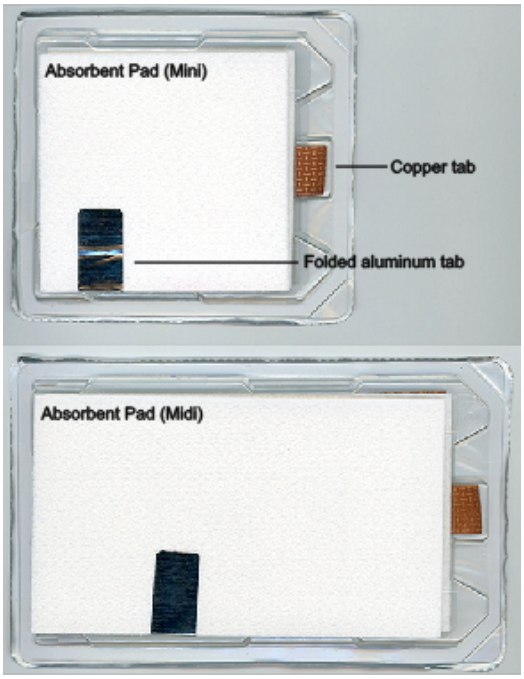
iBlot™ Filter Paper

The iBlot™ Filter Paper is supplied in two sizes for efficient blotting of mini or midi gels. Briefly wetted in deionized water, 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.

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™ 3 Absorbent Pad

The iBlot™ 3 Absorbent Pad absorbs any excess liquid formed during blotting and generates even pressure on the stack assembly. The aluminum tab on each side of the pad acts as an electrical contact between the assembled stack and the cathode plate.



When properly assembled, the aluminum tab of the iBlot™ 3 Absorbent Pad is placed away from the copper tab and allows completion of the electrical circuit.

Note: The folded aluminum tab on the absorbent pad can be placed in other orientations, however, for optimal results use the recommended orientation.

Discard the iBlot™ 3 Absorbent Pad after every use. Do not reuse the iBlot™ 3 Absorbent Pad.

Operating the iBlot™ 3 Western Blot Transfer Device

First time usage of the iBlot™ 3 Western Blot Transfer Device

The first time the iBlot™ 3 Western Blot Transfer Device is turned on, you will need to perform the following actions:

1. Accept the End User License agreement.
2. Configure **Date & Time** including **Time Zone**, **Date/Format**, and **Time/Format** by following the screen prompts.
3. Change the **Instrument Name** if desired.

Control panel of the iBlot™ 3 Western Blot Transfer Device

The control panel is a touch screen display allowing the user to run the transfer method and access device settings.

1. Press **Settings**. The **Settings** screen allows the user to perform the following actions:

About Instrument	Instrument Settings	Maintenance & Services
• About instrument • End User License Agreement (EULA) • Check updates	• Instrument name • Date & time • Sleep mode • Brightness • Buzzer control	• Firmware update • Restore factory settings • Self verification test • For factory use
Run Log	**Error Log**	**Methods Management**
• Method name, Station, Date (up to 250 run logs) • Export run log	• Date, Error description • Export error log	• Import, Export, or Delete user's method • Change method parameters and rename

2. Run the method.
   - Run a preset template (default pre-programmed method).
   - Run the method last used on the instrument.
   - Run a custom method.

Set the date and time

1. Press **Accept** on the End User License Agreement screen to access the **Date & Time** screen.
2. Touch the **Date & Time** screen to modify the Time Zone.
3. Touch the fields to choose a date format, then press **Enter**.
4. Set the date display in Short or Long version. Press **Done** to return to **Date & Time** screen.
5. Touch the fields to choose a time format (12- or 24-hr format).
6. Touch **Done** to return to the **Date & Time** screen. Press **Next** to access the **Instrument Name** screen.
7. If desired, input the personal instrument name using the keyboard and press **Enter**.
8. Press **Done** to continue to the **Set Up Run** screen.

Write logs to a USB storage device

A record of each run is kept by the iBlot™ 3 Western Blot Transfer Device with transfer parameters (voltage, current, time) and temperature being tracked at 1-second intervals. This information can be downloaded to a USB storage device in a .csv format.

1. Insert a USB storage device into the USB port (Type A) located on the right side of the device.

   Note: On average, the time to detect the USB drive once inserted is less than 10 seconds. USB detection times vary depending on the USB drive.

2. Touch **Run log** on the **Settings** screen and select a single or multiple methods. Press **Export**.
3. Remove the USB storage device when the "Run log has been exported successfully" message displays.

Description of methods

Methods

The iBlot™ 3 Western Blot Transfer Device is pre-programmed with 3 transfer methods using different combinations of voltage, time, and cooling.

Transfer Method	Run Parameters
Broad Range (30–250 kDa)	25 V, 6 mins, Low Cooling
High Molecular Range (150–400 kDa)	30 V, 8 mins, No Cooling
Low Molecular Range (10–120 kDa)	25 V, 3 mins, Medium Cooling

- Broad Range – Recommended for standard gel types and optimized for transfer of proteins between 30–250 kDa.
- High Molecular Range – Recommended for Tris-Acetate gels and optimized for transfer of proteins between 150–400 kDa.
- Low Molecular Range – Recommended for standard gel types and Tricine gels and optimized for transfer of proteins between 10–120 kDa.

Recommended running parameters

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.

Table 1 Additional recommended parameters for protein transfer with a molecular weight range of 30–250 kDa

Transfer Stack	Voltage	Transfer Time	Cooling
Midi transfer stacks (2 mini gels or 1 midi gel)	25–30 V	5–7 mins	No cooling or Low cooling
Mini transfer stack (one mini gel)	25 V	5–6 mins	Low cooling or Medium cooling

Table 2 Additional recommended parameters for protein transfer with a molecular weight <30 kDa. Cooling setting is gel dependent. No cooling or low cooling is recommended for Tricine gels, but some optimization is required.

Transfer Stack	Volts	Transfer Time	Cooling
Midi transfer stacks (2 mini gels or 1 midi gel)	20–25 V	3–5 mins	Low cooling or Medium cooling
Mini transfer stack (one mini gel)	25 V	2–4 mins	Low cooling or Medium cooling

Table 3 Additional recommended parameters for protein transfer with a molecular weight >150 kDa

Transfer Stack	Volts	Transfer Time	Cooling
Midi transfer stacks (2 mini gels or 1 midi gel)	25–30 V	8–10 mins	No cooling
Mini transfer stack (one mini gel)	25–30 V	6–8 mins	No cooling

Based on your initial results, blotting parameters may need to be optimized (see “Optimizing blotting” on page 37). Parameters may include:

- Increasing or decreasing the transfer time.
- Setting cooling to low, medium, maximum, or no cooling.
- Performing a water wash gel equilibration step prior to transfer.

2 Protein transfer protocol

Experimental overview

Experimental outline

The table below outlines the experimental steps necessary to perform western blotting using the iBlot™ 3 Western Blot Transfer Device. For more details on each step, see indicated pages.

Step	Action	Page
1	Select Method for performing transfer.	"Select a method" on page 24
2	Assemble the iBlot™ 3 Transfer Stack with your pre-run gel for transfer using the iBlot™ 3 Western Blot Transfer Device. **Note:** Depending on the target protein and gel type, a 5-minute deionized water wash is recommended prior to transfer.	"Assemble the iBlot™ 3 Transfer Stack" on page 24
3	Perform protein transfer using the Pre-programmed or Custom Method.	"Perform blotting" on page 26
4	Disassemble the iBlot™ 3 Transfer Stack.	"Disassemble the iBlot™ 3 Transfer Stack" on page 26

General guidelines

To obtain optimal results, follow these recommendations:

- For the recommended gel type or high molecular-weight target protein, perform a wash step for 5 minutes using 50–100 mL of deionized water.
- Cut off the gel foot and well fingers to ensure even gel thickness across the transfer stack. For best results, cut the gel as close to the bottom of the wells as possible while ensuring the top of the gel is straight and even after cutting. This is especially important for WedgeWell™ gels.
- Wear gloves at all times during the entire blotting procedure to prevent contamination of gels and membranes. If you need to adjust the membrane, always use forceps.

  Note: In some instances, the membrane may adhere to the bottom of the divider. If the membrane is stuck on the divider, use forceps to remove the membrane and place it on top of the Bottom Stack, then gently roll with the roller to ensure good contact between the membrane and stack. PVDF membrane may require a brief 100% methanol rehydration followed by deionized water wash before placing the membrane back on the Bottom Stack.

- Do not use expired iBlot™ 3 Transfer Stacks.

- Remove air bubbles using the Blotting Roller supplied with the device.
- Do not allow the stack to stay in the device for more than 5 minutes after the transfer. Excess time in the device can cause membrane discoloration and higher background.
- Do not trim the membrane or iBlot™ 3 Transfer Stacks to fit your gel size. Maintain the membrane size identical to the transfer stacks to avoid direct contact between the top and bottom transfer stacks.
- Gently close the lid after assembling the transfer stacks to ensure metal contacts and transfer stacks do not shift out of position.
- Wipe down the instrument, cathode plate, and contacts after every use.

Recommended gel types

The gel types compatible for use with iBlot™ 3 Western Blot Transfer Device and iBlot™ 3 Transfer Stacks are listed below.

Gel Type	Size	iBlot™ 3 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	Midi, NC Midi, PVDF
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 mm or 1.5 mm thick	Mini, NC Mini, PVDF Midi, NC Midi, PVDF

Using the iBlot™ 3 Western Blot Transfer System

Materials needed

You will need the following items:

- Pre-run mini or midi gel containing your protein samples and standards
- iBlot™ 3 Transfer Stack, Midi for blotting one midi gel or two mini gels
  OR
- iBlot™ 3 Transfer Stack, Mini for blotting one mini gel
- Blotting Roller supplied with the device
- Tray with deionized water for rinsing and washing the gel
- Tray with deionized water for wetting the filter paper
- Forceps

Select a method

Select the appropriate Method for your application on the iBlot™ 3 Western Blot Transfer Device prior to assembling an iBlot™ 3 Transfer Stack with your gel.

1. Press the power switch at the rear of the device (“Device views” on page 8) to turn ON the device.

   Note: When powering ON the device, keep the device lids closed to avoid a **Power On Test Failure**.

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

2. Touch **Set up run** to select either station.

3. Select Station 1, Station 2, or both stations and touch **Set up selected**.

4. Select the method.

   - **All**
   - **Pre-Programmed Methods**
   - **Custom Method**

   Note: The **Custom Method** tab will not be visible during the first-time use.

Assemble the iBlot™ 3 Transfer Stack

1. Open the lid of the device using the handle.

2. Ensure the blotting surface and cathode plate are clean.

3. Unseal the iBlot™ 3 Transfer Stack by pulling the top right corner of the seal.

   A white plastic divider separates the Top Stack from the Bottom Stack.

4. If using iBlot™ 3 Transfer Stacks, Low Fluorescence PVDF, you must activate the membrane before use. To activate the membrane, go to Step 5.

   If using iBlot™ 3 Transfer Stacks other than Low Fluorescence PVDF, the membrane does not need to be activated. Go to Step 6 to continue with the assembly procedure.

5. To activate the iBlot™ 3 Transfer Stack Low Fluorescence PVDF membrane, remove the membrane from the pouch (included in box of transfer stacks) and incubate it in 100% methanol or 100% ethanol for 3 minutes. Rinse the activated membrane in deionized water before placing it on top of Bottom (anode) Stack.

6. Keep the Bottom Stack in the transparent plastic tray and place the divider with the Top Stack on the bench with the copper electrode facing up.

   Note: In some instances, the membrane may adhere to the bottom of the divider. If the membrane is stuck to the divider, use forceps to remove the membrane and place it on top of the Bottom Stack, then gently roll with the roller to ensure good contact between the membrane and stack.

PVDF membrane may require a brief 100% methanol rehydration followed by deionized water wash before placing the membrane back on the Bottom Stack.

7. After completion of electrophoresis, remove the gel from the cassette. Cut off the gel foot (if present) and well fingers and immerse the gel briefly in deionized water (5–15 seconds) to facilitate easy positioning of the gel on top of the transfer membrane.

   In general, there is no need for any pretreatment of the gel after electrophoresis, however, some gel types and proteins show enhanced transfer efficiency after pretreatment.

   - For WedgeWell™ Tris-Glycine and Novex™ Tris-Glycine Plus gels, we recommend an equilibration in 50–100 mL of deionized water for 5 minutes prior to transfer.
   - When transferring high molecular-weight proteins (>150 kDa) for all gel types and chemistries, we recommend a 5-minute equilibration step in deionized water.
   - The transfer membrane is supplied in a ready-to-use format in the stacks without any need for pretreatment. PVDF membrane is preactivated prior to assembly with the transfer stack, so no additional treatment is required. However, if PVDF membrane adheres to the divider, a brief 100% methanol rehydration followed by a deionized water wash may be required before placing the membrane on the Bottom Stack.

   Note: Use the appropriate iBlot™ 3 Transfer Stack based on the gel size used for blotting. Do not trim the membrane or transfer stack to fit the size of the gel, as the transfer quality is not affected if the pre-run gel is smaller than the transfer stack. Always maintain the membrane size to be the same size as the transfer stack to avoid accidental contact between the bottom (anode) and top (cathode) parts of the stack.

8. Shake off any excess water from the pre-run gel and place on the transfer membrane of the Bottom Stack as described:

   - 1 midi gel on an iBlot™ 3 Midi Transfer Stack
   - 2 mini gels (with wells facing each other) on an iBlot™ 3 Midi Transfer Stack
   - 1 mini gel on an iBlot™ 3 Mini Transfer Stack

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

   Note: Gently roll the gel from the wells side towards the dye-front (north-to-south) to avoid over-stretching and distorting the gel lanes sideways.

9. Briefly pre-wet one filter paper in deionized water and place on top of the gel.

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

10. Take the Top Stack from the bench and place it on top of the pre-wetted filter paper with the copper electrode facing up (and transfer gel layer facing down and in contact with the membrane). **Discard the white plastic divider.**

11. Place the tray with the assembled stack layers directly on the blotting surface. Align the copper tab of the tray with the ⊕ symbol (two possible locations per each station). Do not push the tray too hard as this may elevate and twist the tray or interfere with the contacts. The copper tabs on the tray should be aligned with the corresponding electrical contacts on the lid of the iBlot™ 3 Western Blot Transfer Device.

    Gently roll with the Blotting Roller on top of the Top Stack copper to ensure good contact between all layers of the assembled stack.

12. Place the iBlot™ 3 Absorbent Pad on top of the assembled stack such that the longer side of aluminum tab is facing up and it is further away from the copper tab.

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

Perform blotting

After assembling the iBlot™ 3 Gel Transfer Stack, perform blotting as described below. Perform blotting within 5 minutes of assembling the stacks with the gel.

1. **Gently close the lid by first pressing down the handle, then pulling the handle forward.**

    Note: Do not forcibly push the lid when closing. This can cause the transfer stack or metal contacts to shift out of position, or it could cause breakage of the handle contacts.

2. Select the transfer method and press **Start run** to begin the transfer.

3. At the end of the transfer, the current automatically shuts off and the device signals the end of the transfer with alert sounds (if set up in Settings) and a message on the display.

4. Touch **Done**.

5. Proceed to “Disassemble the iBlot™ 3 Transfer Stack” on page 26.

Disassemble the iBlot™ 3 Transfer Stack

To obtain good transfer and detection results, open the device and disassemble the stack within 5 minutes of ending the blotting procedure.

1. Lift the handle up to open the device lid.

2. Discard the iBlot™ 3 Absorbent Pad and Top Stack.

    Note: Occasionally the absorbent pad may stick to the stainless-steel cathode plate. Remove and discard the pad.

3. Carefully remove and discard the transferred gel and filter paper.

4. Remove the transfer membrane from the stack and proceed with downstream processing or post-transfer analysis (see “Post transfer analysis” on page 36 for details).

Note: PVDF membrane dries quickly. Upon removal from the stack, immediately place the membrane into deionized water. If the PVDF membrane is dry, re-wet with 100% methanol for 10–15 seconds to ensure complete, even rehydration, and rinse with deionized water several times before use. Transfer the membrane to the blocking or staining solution only after confirming the membrane is completely wet. Reactivating after the membrane is exposed to the blocking solution may be problematic.

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5. Remove and discard the plastic tray containing the Bottom Stack.

6. Gently wipe down the instrument and metal contacts with a damp cloth or paper tissue to remove any excess liquid that may not have been absorbed by the iBlot™ 3 Absorbent Pad.

7. At this point, the iBlot™ 3 Western Blot Transfer Device is ready for another run (cooling fan automatically turns on after each run for temperature consistency between runs). If you are not using the device, leave it in the idle position or turn off the power switch located on the back of the device.

IMPORTANT! Do not reuse the iBlot™ 3 Absorbent Pad, iBlot™ Filter Paper, or Top and Bottom Stacks after blotting. Discard after each use.

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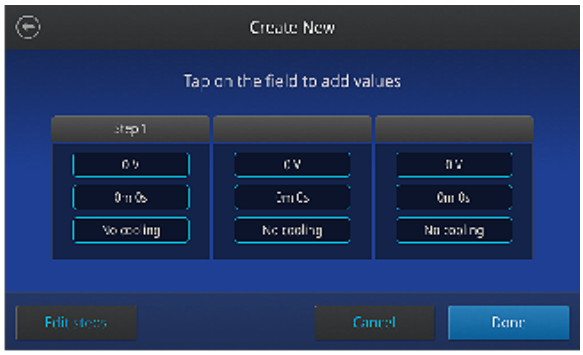
3 Custom methods

Create custom methods

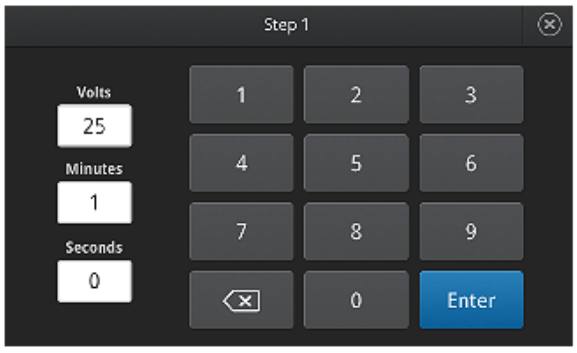
Create a new method

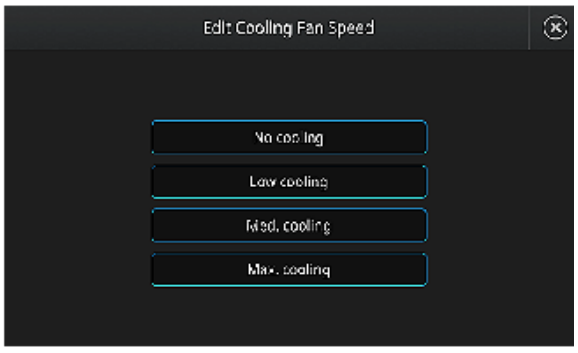
Custom methods can be created and saved on the iBlot™ 3 Western Blot Transfer Device for specific applications or fine-tuning transfer conditions. Custom Methods can be created by editing a pre-programmed method or by creating a new method. There is no limit to the number of custom methods that can be created.

1. On the **Set up run** screen, select **Station 1**, **Station 2** or both and press **Set up selected**.
2. On the **Select Method** screen, press **Create New**. The default screen with **0 V**, **0m 0s**, and **No cooling** appears.



3. Tap the desired field and use the keyboard to enter the appropriate values for **Volts** (5 V to 35 V), **Time** (1 min to 25 mins), **Cooling fan speed** (No cooling, Low cooling, Med. cooling, or Max. cooling) for step 1. Repeat the procedure as necessary for step 2 and step 3.

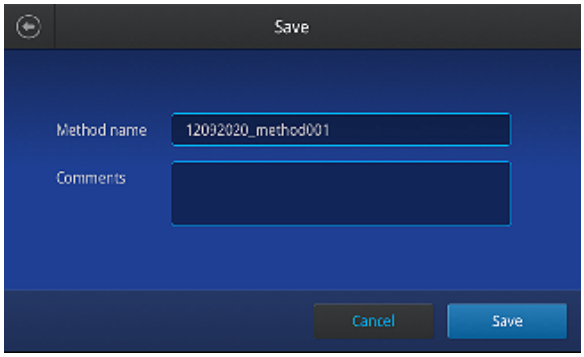




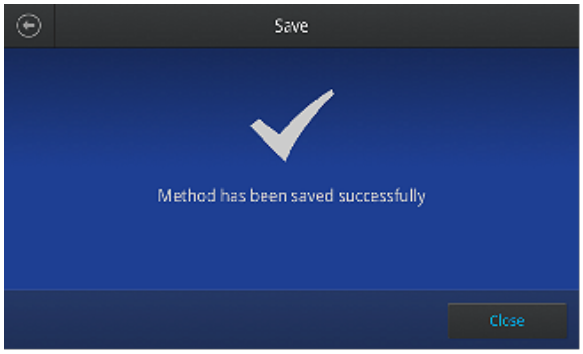
4. Touch **Edit steps** to add steps or remove steps. Up to 10 steps can be added to each method.

5. After adding the voltage, time, and cooling parameters in each step, touch **Done**.

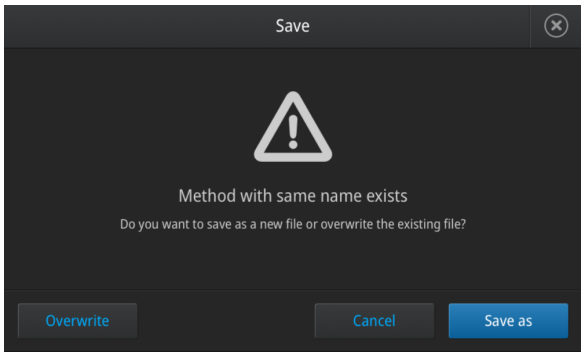
6. Save the newly created custom method by entering the **Method name** and method description in the **Comments** field, and touch **Save**.



7. Touch **Close**.



Note: If the name with the same file name exists, a warning message will appear to save the method as a new file or overwrite the existing file.



8. Touch **Start run** to begin the transfer.

The new saved method will be listed in **All** and **Custom Methods**.

Edit existing methods

Custom methods can be created and saved on the iBlot™ 3 Western Blot Transfer Device for specific applications or fine-tuning transfer conditions. Custom Methods can be created by editing a pre-programmed method or by creating a new method.

1. On the **Set up run** screen, select **Station 1**, **Station 2** or both and press **Set up selected**.
2. Choose and open an existing method from pre-programmed or saved custom methods.
3. Select a **Pre-programmed** or **Custom method**. Touch **(Details)** to see the method name, details, and description.

Note: For a pre-programmed method, "Template" will be added to the method name.

---

4. Tap the desired field and use the keyboard to enter the appropriate values for **Volts** (5 V to 35 V), **Time** (1 min to 25 mins), **Cooling fan speed** (No cooling, Low cooling, Med. cooling, or Max. cooling). Press **Enter**.

Note: Choosing the optimal cooling setting depends on the molecular weight of the target protein, transfer time, and voltage. In general, high molecular proteins transfer best with higher transfer temperature (**No cooling**). Low molecular proteins transfer better with lower temperature (**Low cooling** or **Med. cooling**), which slows down the transfer and decreases blow-through. Medium molecular weight proteins should generally be transferred with **No cooling** or **Low cooling**. When increasing transfer time and/or voltage, some cooling is recommended. For additional cooling recommendations, see the tables in “Methods” on page 20 and “Recommended running parameters” on page 20.

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5. Touch **Edit steps** to add steps or remove steps. Up to 10 steps can be added to each method.

Note: Maximum duration for the method is 25 minutes. If the method exceeds the maximum time at the selected voltage, the total time has to be adjusted to save or run the method.

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6. Touch **Done** to confirm and exit edit mode.

7. Touch **Start run** without saving or **Save as**.

   Note: Pre-programmed methods cannot be overwritten. If the method already exists, then use the option to save as a new file or overwrite the existing file. If transferring without saving, an Unsaved Method name will appear above the time dial. At the end of the run, the option to save the method will appear.

8. After saving, touch **Start run** to begin the transfer.

9. At the end of the transfer, the current automatically shuts off and the device signals the end of the transfer with repeated alert sounds and a message on the display. Touch **Done** to stop the alert sounds.

10. Disassemble the transfer stack following the instructions in “Disassemble the iBlot™ 3 Transfer Stack” on page 26.

Create custom methods from an existing template (up to 10 steps)

Custom methods can be created and saved on the iBlot™ 3 Western Blot Transfer Device for specific applications or fine-tuning transfer conditions. Custom Methods can be created by editing a pre-programmed method or by creating a new method.

1. On the **Set up run** screen, select **Station 1**, **Station 2** or both and press **Set up selected**.

2. Choose and open an existing method from pre-programmed or saved custom methods.

3. Select a **Pre-programmed** or **Custom method**. Touch **(Details)** to see the method name, details, and description.

   Note: For a pre-programmed method, "Template" will be added to the method name.

4. Tap the desired field and use the keyboard to enter the appropriate values for **Volts** (5 V to 35 V), **Time** (1 min to 25 mins), **Cooling fan speed** (No cooling, Low cooling, Med. cooling, or Max. cooling). Press **Enter**.

5. Touch **Edit steps** to add steps or remove steps. Up to 10 steps can be added to each method.

   Note: Maximum duration for the method is 25 minutes. If the method exceeds the maximum time at the selected voltage, the total time has to be adjusted to save or run the method.

6. After saving, touch **Start run** to begin the transfer.

7. At the end of the transfer, the current automatically shuts off and the device signals the end of the transfer with repeated alert sounds (if set up in Settings) and a message on the display. Touch **Done** to stop the alert sounds.

8. Disassemble the transfer stack following the instructions in “Disassemble the iBlot™ 3 Transfer Stack” on page 26.

A

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the iBlot™ 3 Western Blot Transfer Device and iBlot™ 3 Transfer Stack.

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

Observation	Possible cause	Recommended action
No stack detected	Incorrect placement of the plastic tray leading to interference with contacts.	Ensure that the stack's copper tab is aligned with the ⊕ symbol on the blotting device.
	Plastic divider was not removed when assembling stack.	Make sure that the plastic divider is removed from the stack (see "Assemble the iBlot™ 3 Transfer Stack" on page 24).
	The metal electrical contacts in the lid did not make contact.	Inspect the metal electrical contacts; contacts may need to be replaced (Cat. No. A56427) as part of routine maintenance.
	One or more copper electrical contacts were bent and unable to make good contact with the base unit.	Do not press forcibly on the lid in an attempt to engage the contacts. Excessive force can cause the contacts to shift out of position and damage the unit, or the contacts could break. Contacts may need to be replaced (Cat. No. A56427) as part of routine maintenance.
	The top felt was missing.	To ensure proper contact of the lid and the stack, all stack components must be used and the correct stack thickness maintained.
	The Top Stack was placed on the device upside-down.	Make sure the Top Stack is assembled with the copper electrode facing up.
	The stack was not assembled correctly.	Check your stack, then try again.

Observation	Possible cause	Recommended action
Difficulty closing lid **Note:** Do not press forcibly on the lid as it can cause damage to the unit.	The transfer stack was too thick.	The iBlot™ 3 Western Blot 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 filter paper that is supplied with the iBlot™ 3 Transfer Stack.
		Ensure the stack tray is properly aligned with the ⊕ symbol. Do not remove the transfer stack from the plastic sample tray.
No proteins transferred to the membrane	No current or incorrect method was used.	See "Schematic of iBlot™ 3 Transfer Stack showing the flow of current" on page 10 to ensure the electrical circuit is complete and current is flowing through the device. Be sure to use the correct Method (see "Recommended running parameters" on page 20).
	The transfer stack was assembled incorrectly.	Ensure all stack components are assembled in the correct order and that the divider has been removed during assembly.
	Low Fluorescence PVDF membrane was not activated in 100% methanol or 100% ethanol before use.	Activate the Low Fluorescence PVDF membrane by incubating it in 100% methanol or 100% ethanol for 3 minutes, then rinsing it in deionized water. Ensure that the membrane is evenly wetted.
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 during transfer stack assembly.
	Expired or creased membranes were used.	Use the iBlot™ 3 Transfer Stack before the expiration date printed on the package.
		Ensure the membrane is flat during stack assembly.
High molecular weight proteins remain in the gel indicated by staining of the gel after transfer	Incorrect method or transfer conditions were used. **Note:** It is normal for some proteins to remain in the gel after transfer and is not necessarily a good indicator of performance. Since the iBlot™ 3 Western Blot Transfer System often results in better immunodetection compared to semi-wet and semi-dry transfer apparatuses, complete transfer of proteins is not required. See **thermofisher.com/iblot3**.	Use the appropriate method and run time based on the gel type as described (see "Recommended running parameters" on page 20).
		For mini or midi gels: • Perform a water wash step as described (see "Optimizing blotting" on page 37) to improve transfer. • Use a Tris-acetate gel to separate high molecular weight proteins. • Increase the transfer time in 30-second increments.

Observation	Possible cause	Recommended action
Protein blow-through	Transfer time was too long.	Reduce transfer time by 30-second increments. **Note:** Pre-stained markers use charged dyes and tend to blow-through more than regular proteins.
	Incorrect gel type was used for transfer.	Change the gel type or the membrane type. Proteins can sometimes transfer differently from different gel types and/or to different membranes.
Protein bands distorted on membrane	Non-uniform electric field created around wells.	Ensure the gel is properly flattened using the Blotting Roller. See “General guidelines” on page 22 to obtain good results.
	Too much pressure exerted on the gel.	Ensure that with low percentage gels (for example, Tris-Acetate), no excessive force is used with the Blotting Roller, which can cause the gel to become distorted.
Protein not binding/transferring to membrane (PVDF)	The 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, then rinse in water before placing the membrane back onto to the transfer stack (anode).
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™ 3 Western Blot 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 a 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.
	The stack was left sitting in (or out of) the device after the transfer.	Disassemble the stack within 5 minutes after completion of the transfer.
Green discoloration of membrane edges	Copper ions carried with liquids reached the membrane.	Disassemble the stack within 5 minutes after completion of the transfer.
		These deposits do not interfere with downstream processes. The stained regions can be cut away, but membrane washing typically results in their removal.

Observation	Possible cause	Recommended action
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 (bottom and top) size. 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 any Thermo Fisher Scientific immunodetection kit.

  Note: When using the iBlot™ 3 Western Blot Transfer 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 needed for use, warm to room temperature. Re-wet the membrane with 100% methanol for a few seconds, then rinse the membrane thoroughly with deionized water to remove residual methanol.
- To visualize proteins on the membrane after blotting, use any method for total protein visualization, such as No-Stain™ Protein Labeling Reagent, Coomassie™ Blue R-250, Ponceau S, Amido Black, Pierce™ Reversible Protein Stain Kit, or SYPRO™ Ruby Protein Blot Stain (see “Additional products” on page 44). The iBlot™ 3 Western Blot Transfer Device blotting protocol is compatible with most of the staining methods listed above.

If you do not detect any proteins on the membrane after immunodetection or staining, see Appendix A, “Troubleshooting”. Refer to the manufacturer recommendations for optimizing immunodetection.

The immunodetection profile of proteins transferred using the iBlot™ 3 Western Blot Transfer 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 37 for details) when using the iBlot™ 3 Western Blot Transfer System for the first time with any new combination of antigen and detection reagents.

Optimizing blotting

Optimizing blotting

When using the iBlot™ 3 Western Blot 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 a water equilibration step before transfer

To improve the transfer of high-molecular weight proteins from mini- or midi-NuPAGE™ or Tris-Glycine gels, submerge the gel in 50–100 mL deionized water and equilibrate for 5–10 minutes at room temperature on a shaker before transfer.

Do not equilibrate for longer than 10 minutes, or sensitivity may be reduced. After equilibration, perform transfer using the iBlot™ 3 Western Blot 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 (see “Select a method” on page 24 for details on customizing a Method).

Do not perform transfer for more than the recommended run time limit indicated for each Method (see “Methods” on page 20).

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. Additionally, using the "No cooling" fan speed facilitates the transfer of proteins >150 kDa.

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 3–4 minutes for your transfer using low cooling. Additionally, if longer transfer time is used (5–6 minutes), maintaining the cooling at medium or maximum will slow down the transfer of small proteins.

Near-complete transfer of prestained standard protein bands is observed with the iBlot™ 3 Western Blot 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™ 3 Western Blot Transfer Device, make sure the device is turned off.
- After each use, wipe off the blotting surface and electrodes with a damp cloth or paper tissue.

General maintenance

- To avoid damaging the iBlot™ 3 Western Blot Transfer Device, do not perform any repairs or service other than general maintenance on the iBlot™ 3 Western Blot 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 (“Customer and technical support” on page 53).

Replacing the fuse

Materials required

- Fuses, 12.5 A, Time-Lag T, 250 VAC, 5 × 20 mm (2) (Cat. No. 100031540)
- Safety glasses
- Powder-free gloves
- Screwdriver, flathead

CAUTION! For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the device.

1. Power OFF and then unplug the device.
2. Turn the instrument around so that the back is facing you.

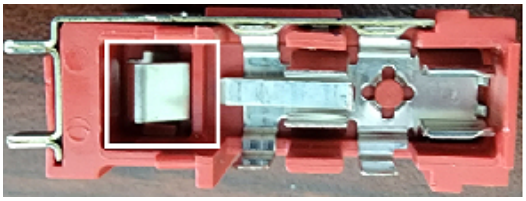
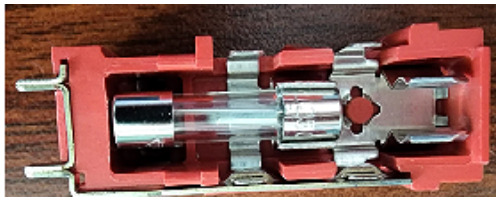
3. Using a small flat-head screwdriver, pry open the fuse door. The door will fall forward from the device.

4. Using the screwdriver, gently pry out the red fuse holder.

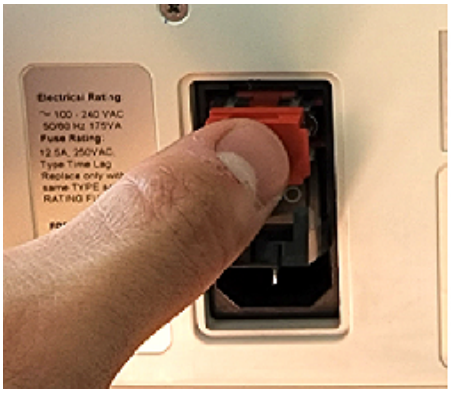
5. Remove each fuse from its fuse holder and inspect it for damage. Black carbon inside the glass typically indicates a defective fuse.

6. Replace each failed fuse with a 12.5 A, Time-Lag T, 250 VAC, 5 × 20-mm fuse.

   Note: The voltage and amperage ratings are on the fuse holder. Ensure the correct orientation of the fuse in the holder (as shown in image below).

7. Re-insert the fuse holder and close the fuse door.



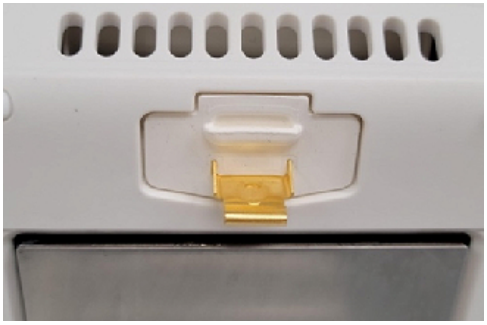

8. Plug in the device, then power ON.

Replacing electrical contacts

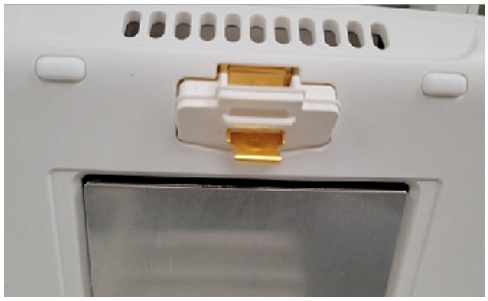
Over time, electrical contacts can become compressed and worn out. Inspect the electrical contacts regularly and replace worn contacts as part of routine maintenance. To ensure optimal life of the electrical contacts, gently wipe down the instrument and contacts with a damp cloth after each use.

To replace an electrical contact (Cat. No. A56427), use a #1 Phillips screwdriver.

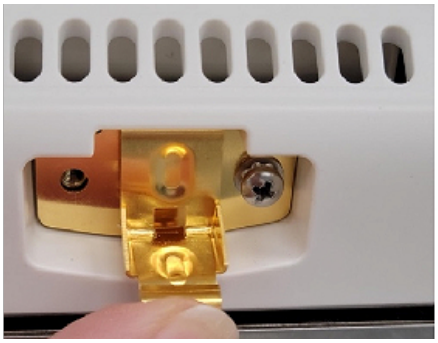
1. Power OFF the device, then open the device lid.

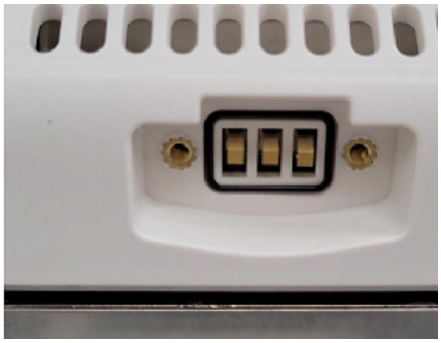


2. Hold the lid with one hand, then pull out the white rubber seal.



3. Using the screwdriver, unscrew the 2 screws holding the contact. Discard the old screw and contact.





4. Clean the area behind the contact with a damp tissue or cloth.

5. Screw in the new contact and replace the rubber seal.

Product specifications

iBlot™ 3 Western Blot Transfer Device specifications

Dimensions:	44 cm (l) × 30 cm (w) × 18.25 cm (h)
Weight:	8.3 kg
Electrical Parameters:	~100–240 VAC, 50/60 Hz, 530VA
Built-in Features:	Digital display, alarm
Compatibility:	Suitable for transfer of mini- (8 × 8 cm), midi- (8 × 13 cm)
iBlot™ 3 Materials:	PC/ABS, Nylon PA12+GF30, Beryllium copper with gold plating, Stainless Steel, EPDM & silicone rubber, Aluminum, Magnesium cast alloy
Operating Temperature:	4–30°C
Blotting Roller:	Delrin™ roller (8.6-cm wide) attached to a stainless steel handle
Replacement Fuse:	10A Slo-Blo, 250V, 5 x 20 mm, (Littelfuse 0218010.MXP)

The iBlot™ 3 Western Blot Transfer Device (plastic enclosure material) is impervious to alcohol, acid (≤ 10% HCl), alkali (≤ 10% NaOH and ≤ 50% DEAE), ≤ 25% Aluminum Oxide but not compatible with acetone, dimethyl sulfoxide, acetic acid, and ≥ 35% concentrated HCl.

The printing ink on the USB and ⊕ icons, are compatible with four common cleaning agents: 10% bleach, 100% IPA (alcohol), 70% ethanol, and mineral oil.

The blue paint around the touch screen area is impervious to five common chemical cleaning agents (10% bleach, 100% isopropyl alcohol, 70% ethanol, <10% HCl, and mineral oil). Using >10% NaOH or 35% HCl will result in color change.

iBlot™ 3 Transfer Stack specifications

Specifications for the iBlot™ 3 Transfer Stacks are listed below. For a more detailed description of the iBlot™ 3 Transfer Stacks, see “iBlot™ 3 Transfer Stacks” on page 44.

Components	
Top Stack	
Midi 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)
Electrode	Copper-coated mesh
Bottom Stack	
Midi Bottom Stack Gel Layer	14.1 cm (l) × 8.5 cm (w) × 0.32 cm (thick)
Mini Bottom Stack Gel Layer	8.5 cm (l) × 8.5 cm (w) × 0.32 cm (thick)
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™ 3 Absorbent Pad	
Dimensions	15 cm (l) × 9.5 cm (w) × 1.1 cm (thick)
Material	Polyester, 10% density
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™ 3 Transfer Stacks

iBlot™ 3 Transfer Stacks are available at **thermofisher.com**. Ordering information is provided below.

Product	Quantity	Cat. No.
iBlot™ 3 Transfer Stack, Midi, Nitrocellulose	1 pack of 10	IB33001
iBlot™ 3 Transfer Stack, Midi, PVDF	1 pack of 10	IB34001
iBlot™ 3 Transfer Stack, Midi, Low Fluorescence PVDF	1 pack of 10	IB34003
iBlot™ 3 Transfer Stack, Mini, Nitrocellulose	1 pack of 10	IB33002
iBlot™ 3 Transfer Stack, Mini, PVDF	1 pack of 10	IB34002
iBlot™ 3 Transfer Stack, Mini, Low Fluorescence PVDF	1 pack of 10	IB34004
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 (1 800 955 6288).

Product	Quantity	Cat. No.
iBind™ Flex Western Device	1 device	SLF2000
iBind™ Flex Cards	10 cards	SFL2010
iBind™ Flex Solution Kit	1 kit	SLF2020
StartingBlock™ (TBS) Blocking Buffer	1 L	37542
Blocker™ FL Fluorescent Blocking Buffer	100 mL	37565
SuperSignal™ West Pico PLUS™ Chemiluminescent Substrate	200 mL	34580
SuperSignal™ West Dura Extended Duration Substrate	100 mL	34075
SuperSignal™ West Femto Maximum Sensitivity Substrate	100 mL	34095
SuperSignal™ West Atto Ultimate Sensitivity Substrate	100 mL	A38555

(continued)

Product	Quantity	Cat. No.
iBright™ Prestained Protein Ladder	2 x 250 µL	LC5615
PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa	2 x 250 µL	26619
MagicMark™ XP Western Protein Standard	250 µL	LC5602
SYPRO™ Ruby™ Protein Blot Stain	200 mL	S-11791
Pierce™ Reversible Protein Stain Kit for Nitrocellulose Membranes	1.5 L	24580
Pierce™ Reversible Protein Stain Kit for PVDF Membranes	1.75 L	24585
No-Stain™ Protein Labeling Reagent	10 reactions	A44717

Precast gels and premade buffers

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

Primary and secondary antibodies

A large variety of primary and secondary antibodies are available at **Thermo Fisher Scientific antibody search tool**.

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, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Hot surface.

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
	Alternating current
	Both direct and alternating current

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

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.

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.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.

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

Cleaning and decontamination

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

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

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™ 3 Western Blot 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.
25	Environmental protection symbol of the China RoHS directive. The number in the symbol indicates the "Environment-friendly Use Period" of the product in years. The symbol is used if a substance restricted in China is used in excess of the maximum permitted limit.
UK CA	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS
TUV	The iBlot™ 3 Western Blot 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.
	Regulatory compliance mark indicating conformity with Australian standards for EMC.

(continued)

Symbol	Information
	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 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
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
EN-61010-1:2010	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 1: General requirements
EN-61010-2-081	Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

EMC standards

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 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
Regulation EC 1907/2006	European Union “REACH” Directive — Registration, Evaluation, Authorisation and Restriction of Chemicals
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**.

Radio compliance standards

Reference	Description
Directive 2014/53/EU	European Union “RE Directive”—Radio equipment
RFID	FCC Notice (for U.S. Customers): This device complies with Part 15 of the FCC Rules: Operation is subject to the following conditions: 1. This device may not cause harmful interference, and 2. This device must accept any interference received, Including interference that may cause undesired operation. Changes and modifications not expressly approved by Thermo Fisher Scientific can void your authority to operate this equipment under Federal Communications Commissions rules.
RFID	Canada: This device complies with Industry Canada licence-exempt RSS standard(s). Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.
RFID	Canada (Français québécois): Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes : (1) l'appareil ne doit pas produire de brouillage, et (2) l'utilisateur de l'appareil doit accepter tout brouillage adioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.