

USER GUIDE

life

Mini Blot Module

Online Specials

For transfer of proteins using the Mini Gel Tank

Catalog Number B1000

Publication Number MAN0007710

Revision A.0

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

life
technologies™

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

Information in this document is subject to change without notice.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

IMPORTANT LICENSING INFORMATION

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS

© 2014 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Contents

Product Contents	4
Description of Parts	5
Before Starting	7
Protein Transfer Protocol.....	13
Maintenance	21
Troubleshooting.....	22
Appendix A	24
Technical Support	24
Related Products	25
Appendix B	26
Buffer Recipes.....	26
Appendix C: Safety	27
Safety Information	27

Product Contents

Mini Blot Module

The Mini Blot Module is a simple apparatus designed for blotting of mini gels and is easily inserted into the Mini Gel Tank in place of a gel cassette assembly. The Mini Blot Module is a semi-wet transfer unit. Each module can be used to perform protein transfer from one mini gel using only 250 mL of 1X transfer buffer.

Product components

The components included with the Mini Blot Module are listed below.

Components	Quantity
Mini Blot Module (consisting of anode core and cathode core)	1 each
Blotting Sponge Pads	4
Blotting Tweezers	1
Blotting Roller	1

Compatibility

The Mini Blot Module can only be used with the Mini Gel Tank. Refer to page 25 for ordering information.

Description of Parts

Specifications

Module Dimensions:	10.5 cm × 14 cm × 6 cm
Blot Module Capacity:	100 mL
Mini Gel Tank:	220 mL/chamber
Blot Size:	8.5 cm × 8 cm

The Mini Blot Module consists of an anode core (+) and a cathode core (-) fitted with a gasket.

Anode core



Anode core, interior



Anode core, exterior

Cathode core



Cathode core, interior

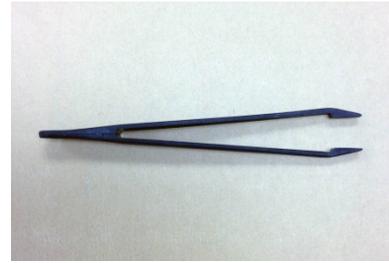


Cathode core, exterior

Description of Parts, Continued

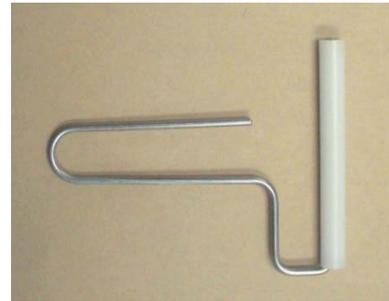
Blotting Tweezers

Blotting Tweezers are supplied for handling transfer membranes.



Blotting Roller

A Blotting Roller is supplied for removing bubbles from the blot sandwich.



Blotting Sponge Pads

Blotting Sponge Pads are included for assembly of the blot sandwich.



Before Starting

- General guidelines**
- Wear the proper protective equipment (gloves, laboratory coat, eye protection) when performing experiments.
 - Prepare 250 mL of 1X Transfer Buffer for each transfer (see page 7).
 - Soak two sponge pads thoroughly in 1X Transfer Buffer (see page 9).
 - Select the type of transfer membrane appropriate for your purpose, and prepare it for transfer (see page 9).
 - Prepare filter paper (see page 10).
 - Trim the wells and the foot from the gel to be transferred (see page 10).
 - Transfer buffer and materials for assembling a blot sandwich can be readied while gel electrophoresis is in progress.
-

- Materials needed**
- Pre-cut blotting membrane and filter paper sandwich
 - Methanol
 - Deionized water
 - Shallow trays for equilibration of membranes, filter paper, and sponge pads
-

**Prepare
1X Transfer Buffer
for Novex® Tris-
Glycine or Tricine
Gels**

For Blotting Novex® Tris-Glycine or Tricine Gels:

250 mL of 1X Transfer Buffer is required for each transfer.

Prepare 1000 mL of 1X Novex® Tris-Glycine Transfer Buffer using Novex® Tris-Glycine Transfer Buffer (25X) as follows:

Reagents	Volume
Novex® Tris-Glycine Transfer Buffer (25X)	40 mL
Methanol*	200 mL
Deionized Water	760 mL
Total Volume	1000 mL

* 1X Transfer Buffer with 10% methanol provides optimal transfer of a single gel in the blot module.

If you are preparing your own transfer buffer, refer to page 26 for a recipe.

Before Starting, Continued

Prepare 1X Transfer Buffer for Bolt® Bis-Tris Plus Gels

For Blotting Bolt® Bis-Tris Plus Gels:

250 mL of 1X Transfer Buffer is required for each transfer.

Prepare 1000 mL of 1X Bolt® Transfer Buffer using Bolt® Transfer Buffer (20X) as follows:

Reagents	Reduced	Non-Reduced
Bolt® Transfer Buffer (20X)	50 mL	50 mL
Bolt® Antioxidant	1 mL	—
Methanol*	100 mL	100 mL
Deionized Water	849 mL	850 mL
Total Volume	1000 mL	1000 mL

* 1X Transfer Buffer with 10% methanol provides optimal transfer of a single gel in the blot module.

If you are preparing your own transfer buffer, refer to page 26 for a recipe.

Prepare 1X Transfer Buffer for NuPAGE® Bis- Tris or Tris- Acetate Gels

For Blotting NuPAGE® Bis-Tris or Tris-Acetate Gels:

250 mL of 1X Transfer Buffer is required for each transfer.

Prepare 1000 mL of 1X NuPAGE® Transfer Buffer using NuPAGE® Transfer Buffer (20X) as follows:

Reagents	Reduced	Non-Reduced
NuPAGE® Transfer Buffer (20X)	50 mL	50 mL
NuPAGE® Antioxidant	1 mL	—
Methanol*	100 mL	100 mL
Deionized Water	849 mL	850 mL
Total Volume	1000 mL	1000 mL

* 1X Transfer Buffer with 10% methanol provides optimal transfer of a single gel in the blot module.

If you are preparing your own transfer buffer, refer to page 26 for a recipe.

Before Starting, Continued

Prepare sponge pads

Add ~200 mL of 1X Transfer Buffer to a container and soak the sponge pads until saturated.

Note: Use clean sponge pads to avoid protein contamination.



Remove air bubbles by squeezing the sponge pads while they are submerged in buffer. Removing air bubbles is essential as they can block the transfer of proteins.



Leave the sponge pads in the container of 1X Transfer Buffer until you are ready to use them to assemble the blot sandwich.

Note: Do not reuse 1X Transfer Buffer used to soak sponge pads. Discard in an appropriate hazardous waste container.

Prepare transfer membrane

Use Novex[®] pre-cut membrane/filter paper sandwiches (see page 25 for ordering information) or cut selected transfer membrane to the dimensions of the gel.

Always handle the membrane with Blotting Tweezers to avoid contamination. **Do not** touch the membrane with bare hands.

- **PVDF membrane**

1. Pre-wet the PVDF membrane for 30 seconds in a tray containing methanol, ethanol, or isopropanol.
2. Briefly rinse membrane in deionized water.
3. Submerge membrane in a shallow tray containing 1X Transfer Buffer for several minutes.

- **Nitrocellulose/Nylon membrane**

Submerge membrane in a shallow tray containing 1X Transfer Buffer for several minutes.

Before Starting, Continued

Prepare filter paper

Use Novex® pre-cut membrane/filter paper sandwiches (see page 25 for ordering information) or cut the required pieces of filter paper to the dimensions of the gel.

Set the filter paper aside until you are ready to use them to assemble the blot sandwich.

Prepare gel for transfer

Remove the gel from the cassette, and prepare it for transfer as described below.

- Prepare the gel for transfer as soon as possible after completion of electrophoresis.
- **Do not touch the gel with bare hands.**
- **To prevent the gel from drying out, do not open the gel cassette until you are ready to assemble the blot sandwich.**

1. Rinse gel cassette with deionized water.
2. Carefully insert the beveled edge of the Gel Knife into the narrow gap between the two plates of the cassette.

Note: Do not push the knife forcefully between the cassette plates or you may cut into the gel.

3. Gently lever the knife up and down to separate the plates. You will hear a cracking sound as the bonds which hold the halves together break. Repeat until you have broken the bonds on one side.



4. Rotate the cassette and repeat Steps 2–3, until the two plates are completely separated.
5. Separate the plates and carefully remove the plate that the gel is not attached to.

Note: The gel may adhere to either plate of the cassette upon opening. If the gel adheres to the short plate, you may need to push the “foot” of the gel out of the slot on the long plate using a Gel Knife for full separation.

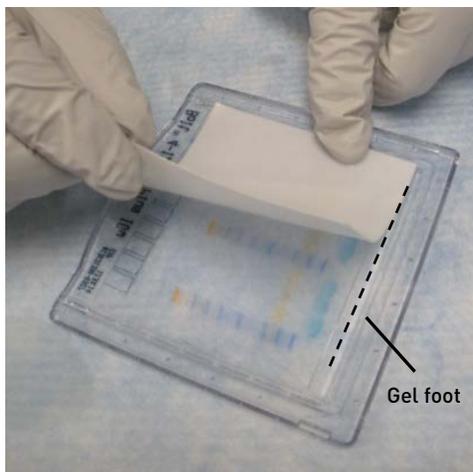
6. Using the Gel Knife, carefully trim off the gel wells.



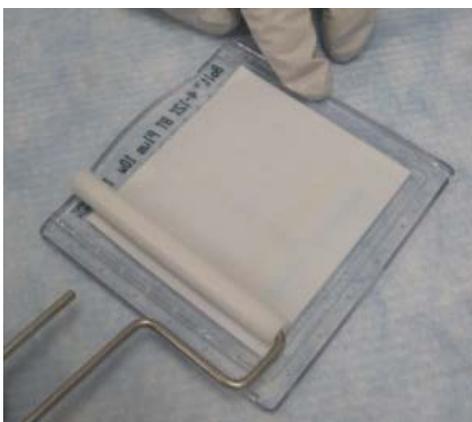
Before Starting, Continued

Prepare gel for transfer, continued

7. Briefly wet a piece of filter paper in 1X Transfer Buffer and place it on top of the gel, just above the “foot” at the bottom of the gel.



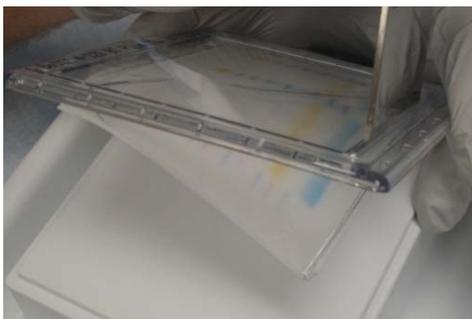
8. Remove any bubbles with the Blotting Roller.



9. Invert the plate over your hand or a hard flat surface and separate the gel from the plate.

- **Gel on long (slotted) plate**

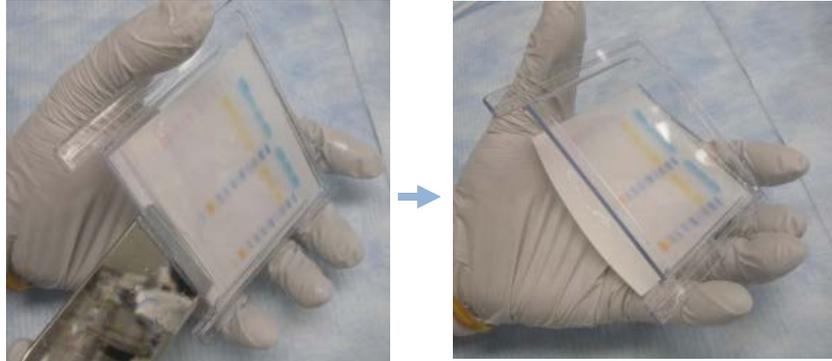
Use the Gel Knife to push the “foot” out of the slot in the plate, and allowing the gel to peel away from the plate.



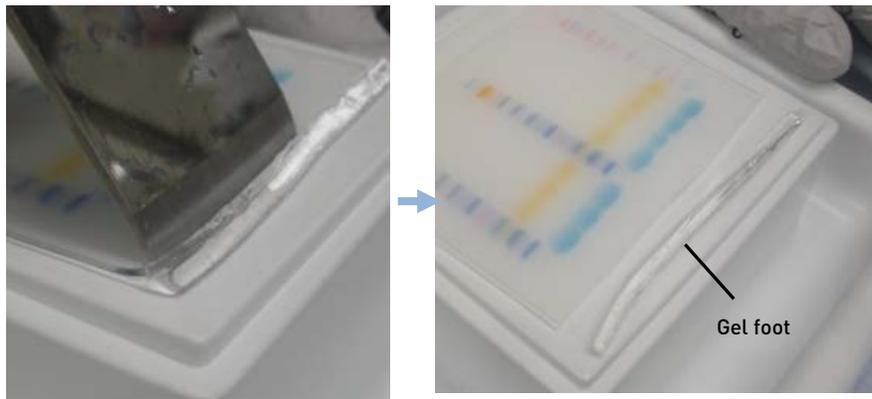
Before Starting, Continued

Prepare gel for transfer, continued

- **Gel on short plate**
Use the Gel Knife to carefully separate the bottom of the gel and allow the gel to peel away from the plate.



10. With the gel on a flat surface, cut the foot off of the gel.



11. Leave the gel on the surface until you are ready to use it to assemble the blot sandwich. **Do not** soak the gel in transfer buffer.
-

Protein Transfer Protocol

Introduction

The following blotting protocol is suitable for majority of protein blotting applications using the Mini Blot Module.

Materials needed

- Previously electrophoresed mini gel (maximum gel size 8.5 cm × 7.5 cm)
 - Mini Blot Module
 - Mini Gel Tank
 - 1X Transfer Buffer
 - Membrane
 - Sponge Pads
 - Blotting Roller
 - Filter Paper
-

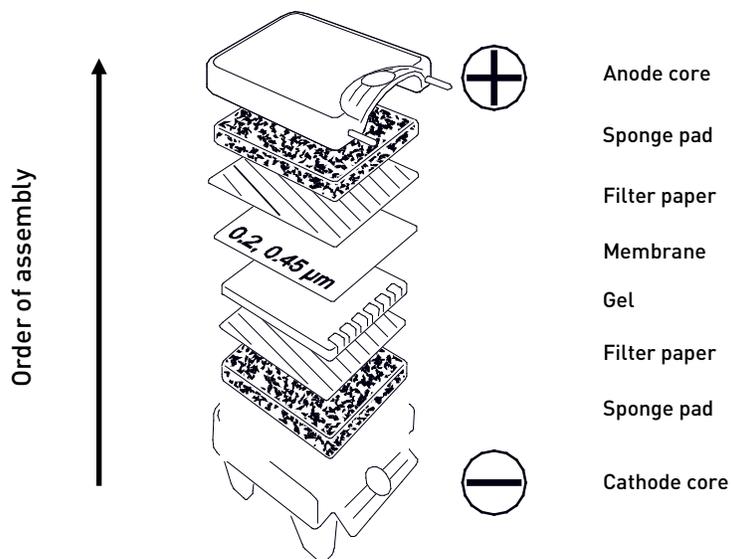
Guidelines

- Only one gel can be transferred at a time in a single blot module
 - One blot module can be used in each chamber of the electrophoresis tank
 - Make sure any Cassette Clamps are removed from the tank before inserting blot modules
 - Always handle the membrane with the Blotting Tweezers.
 - During assembly of the blot sandwich, make sure that sponge pads, filter paper, gel, and membrane do not ride up on the side of the cathode core. If this occurs during assembly, use the Blotting Roller to gently reposition the piece so that it is flush with the other pieces.
 - Use the Blotting Roller to remove any bubbles between layers of the blot sandwich.
-

Protein Transfer Protocol, Continued

Overview

IMPORTANT! When the blot sandwich is fully assembled, the **gel is closer to the cathode core (-)**, while the **membrane is closer to the anode core (+)**.



Assemble blot sandwich and module

Follow the instructions to assemble a blot sandwich for protein transfer.

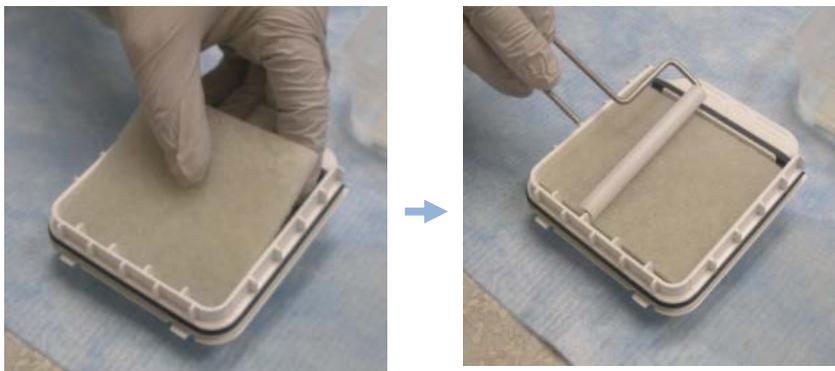
1. Place the cathode core (-) on a flat surface.
2. Add 5–10 mL of 1X Transfer Buffer to the cathode core (-).



Protein Transfer Protocol, Continued

Assemble blot sandwich and module, continued

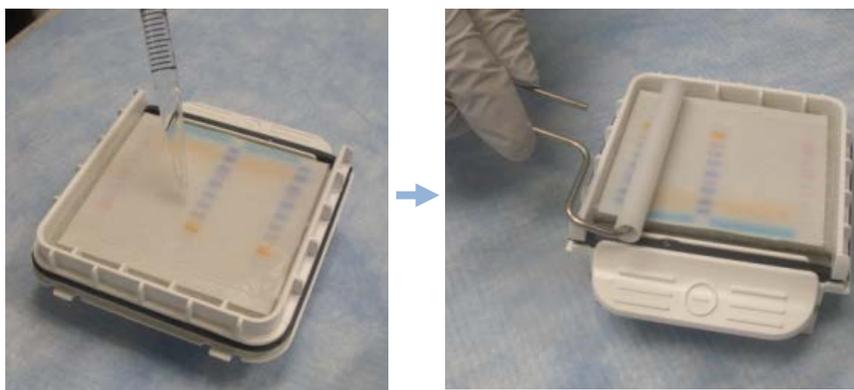
3. Place a soaked sponge pad into the cathode core (-) of the blot module. Remove any bubbles with the Blotting Roller.



4. Take the gel with filter paper (from "Prepare gel for transfer" step 11, page 12) and place it on top of the sponge pad, with the gel side on top.



5. Wet the surface of the gel with 1X Transfer Buffer and remove any bubbles with the Blotting Roller.



Protein Transfer Protocol, Continued

Assemble blot sandwich and module, continued

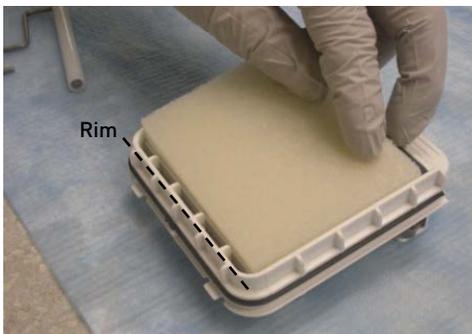
6. Place a pre-soaked transfer membrane on the gel. Remove any bubbles with the Blotting Roller.



7. Wet a piece of filter paper in 1X Transfer Buffer and place it on top of the transfer membrane. Remove any bubbles with the Blotting Roller.



8. Place a pre-soaked sponge pad onto the filter paper. Remove any bubbles with the Blotting Roller.



Note: The sponge pad should rise above the rim of the core. Add an additional sponge pad if necessary. Sponge pads lose resilience and thickness after repeated usage. Replace sponge pads with new ones when resiliency is lost, or if they become discolored.

Protein Transfer Protocol, Continued

Assemble blot sandwich and module, continued

9. Complete the module assembly by placing the anode core (+) on top of the assembled blot sandwich.

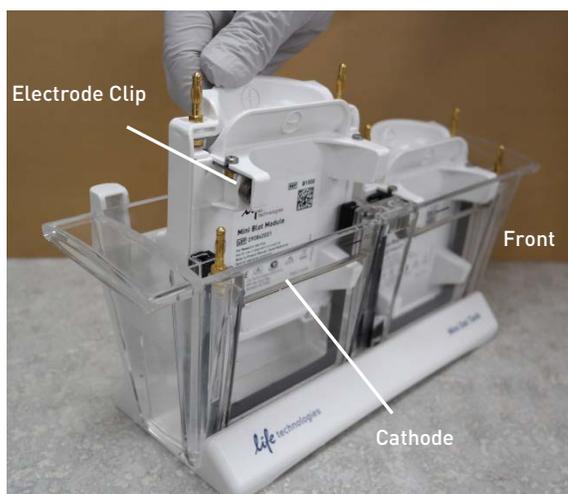


10. Press the two module halves together.



Perform protein transfer

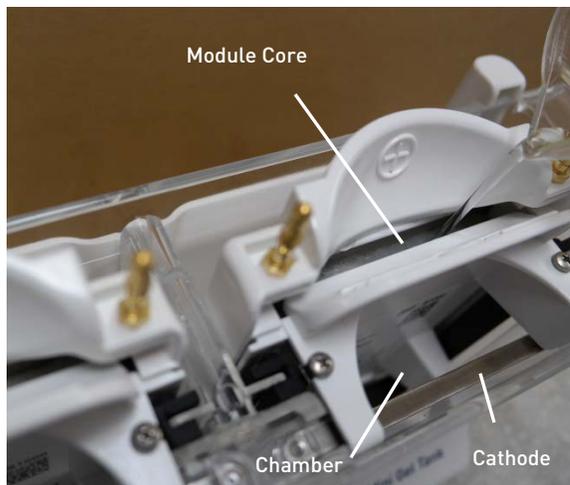
1. Snap the electrophoresis tank into the base. Make sure any Cassette Clamps are removed from the chambers.
2. Insert the blot module with the cathode core (-) facing the front. The blot module should be seated so that the electrodes clips of the module make contact with the electrode bar of the electrophoresis tank.



Protein Transfer Protocol, Continued

Assemble blot sandwich and module, continued

3. If necessary, add 1X Transfer Buffer to the module core to completely submerge the blot sandwich. **Note:** do not fill above the level of the gasket in the blot module.



4. Make sure the power supply is **off** and place the cover on the tank. Push all the way down so that the cover is firmly in place.



5. Plug the power leads into your power supply.
 6. Turn on the power supply, program settings (see "Transfer Conditions") as needed, and start transfer.
-

Protein Transfer Protocol, Continued

Transfer Conditions

Perform protein transfer using the following conditions. Running conditions for voltage and run time are identical whether performing one or two transfers in a single Mini Gel Tank.

Gel Type	Membrane	Voltage (V)	Starting Current (mA)*	Ending Current (mA)*	Run Time
Bolt® Bis-Tris Plus 4-12% (MES)	Nitrocellulose	10	160	60	60
	PVDF	20	340	130	60
NuPAGE® 4-12% Bis-Tris (MES)	Nitrocellulose	10	160	60	60
	PVDF	20	390	130	60
Novex® 4-20% Tris-Glycine (denatured)	Nitrocellulose	10	70	50	60
	PVDF	20	160	100	60
NuPAGE® 3-8% Tris-Acetate (denatured)	Nitrocellulose	10	150	50	60
	PVDF	20	380	130	60
Novex® 10-20% Tricine	Nitrocellulose	10	70	60	60
	PVDF	20	180	150	60

* Current readings represent values when running a single gel, and can vary depending upon the power supply being used.

Note: Faster transfers can be accomplished by using 15 V for 30–45 minutes, however, slight loss of sensitivity may be observed in some cases.

Remove and wash the membrane

1. After protein transfer is complete, turn the power supply off, unplug the power leads, and remove the lid.
2. Remove the blot module from the chamber and empty the transfer buffer into an appropriate hazardous waste disposal container.
3. Open the module assembly.
4. Disassemble the blot sandwich and carefully remove the membrane with Blotting Tweezers.
5. Wash the membrane using 20 mL of ultra-pure water for 5 minutes, two times.

Protein Transfer Protocol, Continued

Post transfer analysis

After the transfer and washing the membrane to remove gel and transfer buffer components and weakly bound proteins, you may 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 from Life Technologies (page 25) or any other immunodetection kit.
 - For storing 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 -20°C or -80°C, as they will shatter.
 - For storing PVDF membranes, air dry the membrane and store the membrane in a 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, followed by thorough rinsing of the membrane with deionized water to remove methanol.
 - For staining the membranes after blotting, you may use:
 - 0.1% Coomassie Blue R-250 in 50% methanol. Do not use Novex[®] Colloidal Blue Staining Kit for staining of membranes, as the background is high.
 - 20 mL of SimplyBlue[™] SafeStain with dry PVDF membranes and incubate for 1–2 minutes. Wash the membrane three times with 20 mL of deionized water for 1 minute. To avoid high background, do not use SimplyBlue[™] SafeStain on nitrocellulose and **wet** PVDF membranes.
 - 0.5% Amido Black in 50% methanol and 10% acetic acid. Remove excess stain with deionized water. Destain with 45% methanol and 10% acetic acid for 30 minutes. Rinse the membrane with deionized water and air dry.
 - 0.1% Ponceau S in 7% trichloroacetic acid (TCA) for 5 minutes. Rinse the membrane in deionized water to obtain transient staining or 10% acetic acid to obtain permanent staining, and air dry.
 - If you do not detect any proteins on the membrane after immunodetection or staining, refer to the **Troubleshooting** section on page 21. Refer to the manufacturer's recommendations for optimizing immunodetection.
-

Maintenance

Cleaning the Blot Module

- Rinse the blot module with deionized water after use.
- To clean any residual build-up in the blot module, apply 50% nitric acid in deionized water to areas inside the blot module until residual build-up is removed.

Once the build-up is removed, rinse the module at least three times in deionized water.

Do not submerge the blot module or soak overnight in nitric acid.

IMPORTANT! Use gloves when preparing the nitric acid solution.

Cleaning the sponge pads

- Clean the sponge pads after each use.
 - Rinse with deionized water and squeeze the water out of the sponge pad 3–5 times.
-

Troubleshooting

Problem	Cause	Solution
No proteins transferred to the membrane	Blot sandwich assembled with gel and membrane in reverse direction such that proteins have migrated into the buffer	Assemble the blot sandwich in the correct order using instructions provided on page 14.
Significant amount of protein is passing through the membrane indicated by the presence of proteins on the second membrane	Longer transfer time, inappropriate SDS or methanol content, or sample overloaded	<ul style="list-style-type: none"> • Re-evaluate the percentage of the gel used. • Shorten the transfer time by 15 minute increments. • Remove any SDS which may have been added to the transfer buffer. • If using nitrocellulose membrane, switch to PVDF which has a higher binding capacity. • Add additional methanol to increase the binding capacity of the membrane. • Decrease the sample load.
Significant amount of protein remains in the gel indicated by staining of the gel after transfer	Shorter transfer time, inappropriate gel type, SDS or methanol content Higher molecular weight proteins usually do not transfer completely as compared to mid to low molecular weight proteins	<ul style="list-style-type: none"> • Switch to a more appropriate lower percentage gel. • Increase the blotting time by 15 minute increments. • Add 0.01–0.02% SDS to the transfer buffer to facilitate migration of the protein out of the gel. • Decrease the amount of methanol in the transfer buffer.
The pH of the transfer buffer deviates from the required value by 0.2 pH units	Buffer not made up properly	Remake the buffer after checking the reagents and water quality. Do not adjust the pH with acid or base as this will increase the conductivity of the buffer and result in higher current during transfer.
Current is much higher than the expected start current	Concentrated buffer used	Dilute the buffer as described on page 7.
	Used Tris HCl instead of Tris Base	Check the reagents used to make the buffer and remake the buffer with correct reagents.
Current is much lower than the expected start current	Very dilute buffer used resulting in increased resistance and low current	Remake the transfer buffer correctly.
	The circuit is broken (broken electrode)	Check the blot module to ensure that the electrodes are intact.
	Leak in the blot module indicated by a decrease in the buffer volume in the module	Be sure to assemble the blot module correctly to prevent any leaking.

Troubleshooting, Continued

Problem	Cause	Solution
Power supply shuts off using recommended blotting conditions	High ionic strength of the transfer buffer	Prepare the buffer as described on page 7.
	Power supply is operating at a current close to the current limit of the power supply	Use a power supply with higher limits.
Diffuse bands and swirling pattern on the membrane	Poor contact between the gel and the membrane	Roll over the surface of each layer of the blot sandwich with a glass pipette to ensure good contact between the gel and the membrane. Saturate the blotting pads with transfer buffer to remove air bubbles.
	Under or overcompression of the gel	Add or remove blotting pads to prevent any type of compression of the gel.
Empty spots on the membrane	Presence of air bubbles between the gel and the membrane preventing the transfer of proteins	Be sure to remove all air bubbles between the gel and membrane by rolling a glass pipette over the membrane surface.
	Expired or creased membranes used	Use fresh, undamaged membranes.
Poor transfer efficiency with PVDF	Membrane not treated properly before use	Be sure that the membrane is pre-wetted with methanol or ethanol.
	Poor contact between the membrane and the gel	Use more blotting pads or replace the old blotting pads with new ones.
	Overcompression of the gel indicated by a flattened gel	Remove enough blotting pads so that the unit can be closed without exerting pressure on the gel and the membrane.
High background on western blots	Insufficient blocking of non-specific sites	Increase the blocker concentration or the incubation time.

Appendix A

Technical Support

Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
 - Search through frequently asked questions (FAQs)
 - Submit a question directly to Technical Support (techsupport@lifetech.com)
 - Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
 - Obtain information about customer training
 - Download software updates and patches
-

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Related Products

Additional products

Ordering information for electrophoresis products available separately from Life Technologies is provided below. For detailed information, visit www.lifetechnologies.com or call Technical Support (page 25).

Product	Quantity	Catalog no.
Mini Gel Tank	1 unit	A25977
Blotting Sponge Pads	1 set (of 8)	EI9052
Mini Blot Module Gasket	1 each	B1001
Blotting Tweezers	1 each	B1002
Blotting Roller	1 each	LC2100
Gel Knife	1 each	EI9010
Bolt® Antioxidant	15 mL	BT0005
Bolt® Transfer Buffer (20X)	125 mL	BT0006
Bolt® Transfer Buffer (20X)	1 L	BT00061
Novex® Tris-Glycine Transfer Buffer (25X)	500 mL	LC3675
NuPAGE® Transfer Buffer (20X)	125 mL	NP0006
PowerEase® 300W Power Supply	1 unit	PS0300
Nitrocellulose Membrane (0.2 µm pore size)	20 membrane/filter paper sandwiches	LC2000
PVDF Membrane (0.2 µm pore size)	20 membrane/filter paper sandwiches	LC2002
Invitrolon™ PVDF Membrane (0.45 µm pore size)	20 membrane/filter paper sandwiches	LC2005
WesternBreeze® Chromogenic Western Blot Detection Kit	1 Kit (Anti-Mouse)	WB7103
	1 Kit (Anti-Rabbit)	WB7105
	1 Kit (Anti-Goat)	WB7107
WesternBreeze® Chemiluminescent Western Blot Detection Kit	1 Kit (Anti-Mouse)	WB7104
	1 Kit (Anti-Rabbit)	WB7106
	1 Kit (Anti-Goat)	WB7108
SimplyBlue™ SafeStain	1 L	LC6060
DryEase® Mini-Gel Drying Base	1 base	NI2300

Appendix B

Buffer Recipes

25X Tris-Glycine Transfer Buffer

25X Novex® Tris-Glycine Transfer Buffer is available from Life Technologies (page 25).

1. To prepare 25X Tris-Glycine Transfer Buffer, dissolve the following reagents in 450 mL of deionized water:

	Concentration (1X)	
Tris Base	18.2 g	12 mM
Glycine	90.0 g	96 mM

2. Mix well and adjust the volume to 500 mL with deionized water. The pH of the buffer is 8.3. Do not adjust with acid or base.
 3. Store at room temperature. The buffer is stable for 6 months at 25°C.
 4. For transfer, dilute the 25X 25X Tris-Glycine Transfer Buffer as described on page 7.
-

20X Transfer Buffer

20X Bolt® Transfer Buffer and 20X NuPAGE® Transfer Buffer is available from Life Technologies (page 25).

1. To prepare 20X Transfer Buffer, dissolve the following reagents in 100 mL of deionized water:

	Concentration (1X)	
Bicine	10.2 g	25 mM
Bis-Tris (free base)	13.1 g	25 mM
EDTA	0.75 g	1 mM
Chlorobutanol*	0.025 g	0.05 mM

2. Mix well and adjust the volume to 125 mL with deionized water. The pH of the buffer is 7.2.
3. Store at room temperature. The buffer is stable for 6 months at room temperature.
4. For transfer, dilute the 20X Transfer Buffer as described on page 8.

*Chlorobutanol is used as a preservative in the transfer buffer and is not necessary for efficient transfer of proteins. If you do not have chlorobutanol, you may prepare the buffer without chlorobutanol but the buffer will not be stable for long periods. Use the buffer within 2 weeks.

Appendix C: Safety

Safety Information

Safety

During operation, the Mini Gel Tank must be used with an external DC power supply designed specifically for electrophoresis applications. This power supply must be isolated from ground so that the DC output is floating. The PowerEase™ 300W Power Supply (page 25) meets these requirements. The maximum electrical operating parameters for the Mini Gel Tank are:

Maximum Voltage Limit:	200 VDC
Maximum Power Limit:	100 Watts*
Maximum Operating Temperature Limit:	40°C

* **CAUTION!**: Even though the electrophoresis tank is rated to 500 V, it is not recommended that the Mini Blot Module above 30 V.

The Mini Gel Tank's lid is designed such that if the lid is removed, the electrical connection to the unit will be broken. **Do not attempt to use the electrophoresis tank without the tank lid. Do not use lids from other gel tanks.**

The Mini Gel Tank is designed to meet EN61010-1 Safety Standards. This product is safe to use when operated in accordance with this instruction manual. If this unit is used or modified in a manner not specified in this manual then protection afforded by the unit will be impaired. Alteration of this unit will:

- Void the warranty.
- Void the EN61010-1 safety standard certification.
- Create a potential safety hazard.

Life Technologies is not responsible for any injury or damage caused by use of this unit when operated for purposes which it is not intended. All repairs and service should be performed by Life Technologies.

The Mini Gel Tank is classified as Class II of IEC 536 for protection against electrical shock.

Safety Information, Continued

Product safety compliance

This device complies with the following safety standards:

- IEC 61010-1:2010 (3rd Edition), "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."
 - UL 61010-1, CSA C22.1 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."
-

Installing the instrument

The product may be installed only under the conditions and in the positions specified by Life Technologies.

Operating the instrument

Operation of the product is subject to the conditions described in this manual. The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Life Technologies.

Service operation requirements

In the event of an equipment malfunction, it is the responsibility of the customer to report the need for service to Life Technologies or to one of the authorized agents. For service information, contact Technical Support (page 24).

Electrical safety

The following information on electrical safety must be observed, failing to follow these instruction may result in electric shock, fire and/or serious personal injury or death.

- Prior to switching on the product, ensure that the nominal voltage setting on the product matches the nominal voltage of the AC supply network.
 - If extension cords or connector strips are implemented, they must be checked on a regular basis to ensure that they are safe to use.
 - Never use the product if the power cable is damaged. Check the power cable on a regular basis to ensure that it is in proper operating condition. By taking appropriate safety measures and carefully laying the power cable, you can ensure that the cable will not be damaged and that no one can be hurt by, for example, tripping over the cable or suffering an electric shock.
 - Do not insert the plug into sockets that are dusty or dirty. Insert the plug firmly and all the way into the socket. Otherwise, sparks that result in fire and/or injuries may occur.
 - Unless expressly permitted, never remove the cover or any part of the housing while the product is in operation. Doing so will expose circuits and components and can lead to injuries, fire or damage to the product.
 - Use suitable overvoltage protection to ensure that no overvoltage (such as that caused by a bolt of lightning) can reach the product. Otherwise, the person operating the product will be exposed to the danger of an electric shock.
 - The overvoltage protection should limit the magnitude of the overvoltage surge to 1kV between the any of the power line and ground.
 - Any object that is not designed to be placed in the openings of the housing must not be used for this purpose. Doing so can cause short circuits inside the product and/or electric shocks, fire or injuries.
 - Prior to cleaning the product, disconnect it completely from the power supply. Use a soft, non-linting cloth to clean the product. Never use chemical cleaning agents such as alcohol, acetone or diluents for cellulose lacquers.
-

Safety Information, Continued

Symbols on instrument

The following table describes the symbols displayed on the instrument.

Informational symbols

The symbols used on the Mini Gel Tank are explained below:



The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required.



The Mini Gel Tank 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 C-Tick symbol denotes that the device is compliant with the electromagnetic compatibility (EMC) of the Australian Communications Authority (ACA).



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.



Product catalog number.



Site of manufacture.

For support visit www.lifetechnologies.com/support or email techsupport@lifetechn.com

www.lifetechnologies.com

15 September 2014

