invitrogen™

XCell II™ Blot Module

Catalog no. EI9051

Rev. Date: 14 December 2009
Manual part no. IM9051

MAN0000740

User Manual

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Product Contents and Specifications

Contents

The XCell II™ Blot Module includes anode core, cathode core, and sponge pad (8/pack).

Specifications

The specifications of the XCell II™ Blot Module are:

Cell Dimensions:	14.5 cm × 14 cm × 11 cm
Blot Module Capacity:	200 mL
XCell *SureLock*™ Lower Buffer Chamber Capacity:	600 mL
Blot Size:	9 cm × 9 cm

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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 www.invitrogen.com/weee for collection and recycling options.

Overview

Introduction

Western or immuno blotting is the transfer of separated proteins in a gel to the surface of a thin support membrane matrix. The proteins are bound and immobilized on the membrane. Southern and northern blotting involves the transfer of separated DNA and RNA, respectively, from a gel to a membrane.

To perform a transfer, the gel is layered next to a membrane and placed in a voltage gradient perpendicular to the gel. Negatively charged molecules will migrate out from the gel, move towards the positive electrode, and get deposited on the membrane.

Advantages of Blotting

The advantages of blotting include:

- Removal of gel impurities from the protein or nucleic acid which may hinder further analysis
- Easy access of proteins or nucleic acids on a blot for further analysis
- Faster analysis of proteins on the blots as protein diffusion is minimized
- Shorter staining and destaining time of proteins on the blot as compared to gels
- Multiple successive reprobing of a single blot for analyses after removal of the probe each time
- Convenient way to store separated proteins or nucleic acids for future use

XCell II™ Blot Module

The XCell II™ Blot Module is a simple apparatus designed for blotting of mini-gels and is easily inserted into the XCell *SureLock*™ Mini-Cell in place of the gel/buffer core assembly. The module has rails to guide the unit into the mini-cell. The XCell II™ Blot Module can be used to perform western, Southern, or northern transfer of two mini-gels using only 200 mL of transfer buffer. The XCell II™ Blot Module is a semi-wet transfer unit. An efficient transfer is obtained, as the resistance is constant across the blotting electrodes producing uniform field strength.

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Overview, Continued

Note

The XCell II™ Blot Module can only be used with XCell *SureLock*™ Mini-Cell or XCell II™ Mini-Cell. Refer to page 32 for ordering information.

Blotting Membranes

Invitrogen offers three types of blotting membrane and filter paper sandwiches. Refer to page 32 for ordering information.

- Nitrocellulose for western or Southern blotting
- PVDF (polyvinylidene difluoride) for western blotting
- Nylon for Southern or northern blotting

See the table below for more details on each membrane.

Membrane	Properties	Applications	Pore size	Reprobing
Nitrocellulose	Most widely used membrane for western blotting Good binding capacity Proteins bind to the membrane due to hydrophobic interactions Protein binding capacity: 80 µg/cm^2	Western transfer Amino acid analysis Solid phase assay systems	0.2 µm 0.45 µm	No

Continued on next page

Overview, Continued

Membrane	Properties	Applications	Pore size	Reprobing
PVDF	Higher binding capacity than nitrocellulose Strong hydrophobic character and solvent resistant Physically stronger than nitrocellulose Compatible with commonly used protein stains and immunodetection methods Protein binding capacity:50-150 µg/cm^2	Protein sequencing Western transfer Amino acid analysis Solid phase assay systems	0.2 µm 0.45 µm	Yes
Nylon	Microporous membrane modified with strongly basic charged groups Ideal for binding negatively charged biomolecules such as DNA and RNA Low background for enhanced resolution Membrane is formed around a non-woven polyester fiber matrix which confers high tensile strength, toughness, and flexibility	Southern, northern and western transfers Solid phase immobilization Dry chemistry test strips Enzyme immobilization Gene probe assays	0.45 µm	Yes

Preparing for Transfer

Introduction

You need to prepare the transfer buffer, blotting pads, and blotting membranes before performing the transfer. You may prepare the transfer buffer and materials for transfer while electrophoresis of the gel is in progress.

Materials Needed

- Pre-cut blotting membranes and filter paper sandwiches
- Methanol
- Deionized water
- Transfer buffer (see below)
- Shallow tray for equilibration of membranes, filter paper, and blotting pads

Wear gloves at all times during the entire blotting procedure to prevent contamination of gels and membranes, and to avoid exposure to irritants commonly used in electrophoresis and blotting procedures.

Do not touch the membrane or gel with bare hands. This may contaminate the gel or membrane and interfere with further analysis.

Preparing Transfer Buffer

Prepare the appropriate buffer for your gel type from the recipes given below.

For Blotting Novex® Tris Glycine or Tricine Gels:

Prepare 1,000 mL of 1X Novex® Tris-Glycine Transfer Buffer using Novex® Tris-Glycine Transfer Buffer (25X) as follows:

Novex® Tris-Glycine Transfer Buffer (25X)	40 mL
Methanol	200 mL
Deionized Water	760 mL
Total Volume	1,000 mL

See 30 for a recipe of Tris-Glycine Transfer Buffer, if you are preparing your own transfer buffer.

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Preparing for Transfer, Continued

Preparing Transfer Buffer, continued

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

Prepare 1,000 mL of 1X NuPAGE® Transfer Buffer using the 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	1,000 mL	1,000 mL

*NuPAGE® Transfer Buffer with 10% methanol provides optimal transfer of a single gel in the blot module. If you are transferring 2 gels in the blot module, increase the methanol content to 20% to ensure efficient transfer of both gels.

Refer to page 30 for a recipe of the NuPAGE® Transfer Buffer, if you are preparing your own transfer buffer.

For Blotting Novex® TBE or DNA Retardation Gels:

Prepare 1,000 mL of 0.5X TBE transfer buffer using the Novex® TBE Running Buffer (5X) as follows:

Novex® TBE Running Buffer (5X)	100 mL
Deionized Water	900 mL
Total Volume	1,000 mL

See page 30 for a recipe of the Novex® TBE Running Buffer, if you are preparing your own transfer buffer.

Preparing Blotting Pads

Use ~ 700 mL of transfer buffer to soak the blotting pads until saturated. Remove air bubbles by squeezing the blotting pads while they are submerged in buffer. Removing air bubbles is essential as they can block the transfer of biomolecules.

Continued on next page

Preparing Transfer Membrane and Filter Paper

Use Novex® pre-cut membrane/filter paper sandwiches (see page 32 for ordering information) or cut selected transfer membrane and filter paper to the dimensions of the gel.

- **PVDF membrane**: Pre-wet the PVDF membrane for 30 seconds in methanol, ethanol, or isopropanol. Briefly rinse in deionized water and then place the membrane in a shallow dish containing 50–100 mL transfer buffer for several minutes.
- **Nitrocellulose/Nylon membrane**: Place the membrane directly in a tray containing the transfer buffer for several minutes.
- **Filter paper**: Soak briefly in transfer buffer immediately before using.
- **Gel**: Use the gel immediately following the run (page 11). **Do not soak the gel in transfer buffer**.

Using the XCell II™ Blot Module

Introduction

The blotting protocol described below is suitable for majority of protein blotting applications using the XCell II™ Blot Module. However, some optimization may be necessary by the user to obtain the best results (page 21).

Materials Needed

- Previously electrophoresed mini-gels (maximum gel size 9 cm × 9 cm)
- XCell *SureLock*™ Mini-Cell

Removing the Gel

Remove the gel from the cassette for transfer after completion of electrophoresis as described below. If you are not ready to perform transfer immediately, you may continue electrophoresis of your gel at a low voltage of 5 V. The gel can be left in the unit for a few hours until you are ready to transfer the gel.

1. After electrophoresis, separate each of the 3 bonded sides of the gel cassette by inserting the gel knife into the gap between the cassette's 2 plates. The notched ("well") side of the cassette should face up.
2. Push up and down on the knife handle to separate the plates. Repeat on each side of the cassette until the plates are completely separated.

   Caution: Use caution while inserting the gel knife between the 2 plates to avoid excessive pressure towards the gel.
3. The gel may adhere to either side of plates upon opening the cassette. Carefully remove and discard the plate without the gel The gel remains on the other plate.
4. Remove wells on the gel with the gel knife.
5. Place a piece of pre-soaked filter paper (as prepared on page 10) on top of the gel, and lay just above the "foot" at the bottom of the gel (leaving the "foot" of the gel uncovered). Keep the filter paper saturated with transfer buffer and remove all trapped air bubbles by gently rolling over the surface using a glass pipette.

Continued on next page

Removing the Gel, continued

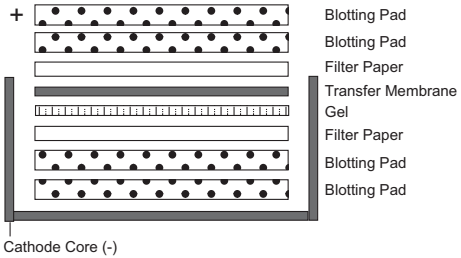
6. Turn the plate over so the gel and filter paper are facing downwards over a gloved hand or clean flat surface covered with a piece of Parafilm®.
7. Remove gel from the plate using the following methods:
   - If the gel rests on the longer (slotted) plate, use the gel knife to push the foot out of the slot in the plate and the gel will fall off easily.
   - If the gel rests on the shorter (notched) plate, use the gel knife to carefully loosen the bottom of the gel and allow the gel to peel away from the plate.
8. When the gel is on a flat surface, cut the "foot" off the gel with the gel knife. Proceed to **Transferring One Gel**, below.

Note: Once you have removed the gel from the unit and the cassette, perform the transfer immediately.

Transferring One Gel

Instructions are provided below for transferring 1 gel. If you need to transfer 2 gels at a time, refer to page 15.

1. Wet the surface of the gel (step 8, above) with the transfer buffer and place pre-soaked transfer membrane on the gel. Remove air bubbles by rolling a glass pipette over the membrane surface.
2. Place the pre-soaked filter paper on top of the transfer membrane. Remove any trapped air bubbles.
3. Place 2 soaked blotting pads into the cathode (–) core of the blot module. The cathode core is the deeper of the 2 cores and the corresponding electrode plate is a darker shade of gray. Carefully pick up the gel membrane assembly with your gloved hand and place on the pad in the same sequence, such that the gel is closest to the cathode plate (see figure below).



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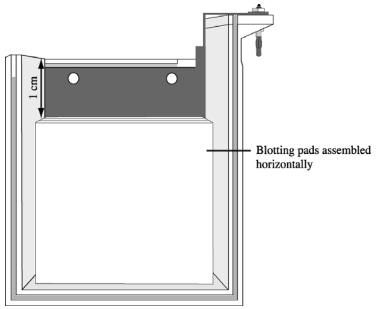
Transferring One Gel, continued

4. Add enough pre-soaked blotting pads to rise 0.5 cm over the rim of the cathode core. Place the anode (+) core on top of the pads. The gel/membrane sandwich should be held securely between the two halves of the blot module ensuring complete contact of all components.

   Note: To ensure a snug fit, use an additional pad since pads lose their resiliency after many uses. Replace pads when they begin to lose resiliency and are discolored.

5. Position the gel membrane sandwich and blotting pads in the cathode core of the XCell II™ Blot Module to fit horizontally across the bottom of the unit. There should be a gap of ~ 1 cm at the top of the electrodes when the pads and assembly are in place (see figure below).



6. Hold the blot module together firmly and slide it into the guide rails on the lower buffer chamber. The blot module fits into the unit in only one way, such that the (+) sign is seen in the upper left hand corner of the blot module. The inverted gold post on the right hand side of the blot module fits into the hole next to the upright gold post on the right side of the lower buffer chamber.

Continued on next page

Transferring One Gel, continued

7. Depending on the mini-cell that you are using, follow the appropriate instructions for positioning the wedge:
   - For XCell *SureLock*™ Mini-Cell, place the gel tension wedge such that the vertical face of the wedge is against the blot module. Push the lever forward to lock it into place.
   - For XCell II™ Mini-Cell, place the front wedge (without the screw hole) such that the vertical face of the wedge is against the blot module. Slide in the rear wedge and push it down firmly.

   Note: When properly placed, the rear wedge will not be flush with the top of the lower buffer chamber. There will be a gap between the rear wedge and lower chamber.
8. Fill the blot module with transfer buffer until the gel/membrane sandwich is covered in transfer buffer. **Do not fill all the way to the top as this will generate extra conductivity and heat.**
9. Fill the outer buffer chamber with ~ 650 mL deionized water by pouring in the gap between the front of the blot module and front of the lower buffer chamber. The water level should reach approximately 2 cm from the top of the lower buffer chamber. This serves to dissipate heat produced during the run.

   Note: If you accidentally fill the outer buffer chamber with the transfer buffer, it will not adversely affect the transfer. The liquid in the outer buffer chamber serves as a coolant. We recommend adding deionized water to the outer buffer chamber to avoid any exposure of the mini-cell to methanol as the mini-cell is susceptible to methanol.
10. Place the lid on top of the unit.
11. With the power turned off, plug the red and black leads into the power supply. Refer to **Transfer Conditions** on page 16 for transfer conditions.

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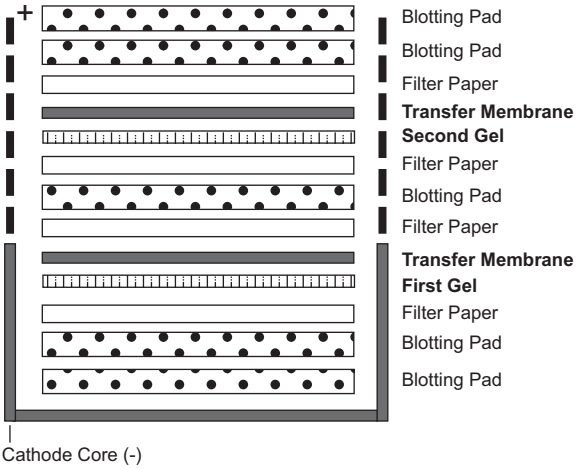
Transferring Two Gels

Instructions are provided below for transferring two gels.

1. Remove the gels after electrophoresis as described on page 11.
2. Assemble the gel/membrane sandwich (as described on page 12) twice to make two gel/membrane sandwiches.
3. Place two pre-soaked pads on cathode core of the blot module. Place the first gel/membrane sandwich on pads in the correct orientation, so the gel is closest to the cathode plate (see figure below).



4. Add another pre-soaked blotting pad on top of the first membrane assembly.
5. Position the second gel/membrane sandwich on top of blotting pad in the correct orientation so that the gel is closest to the cathode side.
6. Proceed with Steps 4–11 as described in **Transferring One Gel** (page 12).

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Transfer Conditions

Choose the transfer conditions from the table below based on type of the gel that you are using.

Note: The expected current listed in the table below is for transferring one gel. If you are transferring two gels in the blot module, the expected current will double. For overnight blotting, see next page.

Type of the Gel	Transfer Buffer (1X)	Membrane	Transfer Conditions	Expected Current
Novex® Tris–Glycine Novex® Tricine	Novex® Tris Glycine Transfer Buffer with 20% methanol. 1X Transfer Buffer should be pH 8.3 before addition of SDS or methanol. Do not adjust the pH.	Nitrocellulose or PVDF	25 V constant for 1–2 hours	Start: 100 mA
NuPAGE® Novex® Bis–Tris	NuPAGE® Transfer Buffer with 10% methanol for transfer of one gel. NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 V constant for 1 hour	Start: 170 mA End: 110 mA
NuPAGE® Novex® Tris–Acetate	NuPAGE® Transfer Buffer with 10% methanol for transfer of one gel. NuPAGE® Antioxidant for reduced samples	Nitrocellulose or PVDF	30 V constant for 1 hour	Start: 220 mA End: 180 mA
Novex® TBE, TBE–Urea, and DNA Retardation	45 mM Tris 45 mM boric acid 1 mM EDTA	Nylon	30 V constant for 1–2 hours	Start: 360 mA End: 270 mA
Novex® IEF*	0.7% acetic acid, pH 3.0, see page 27	Nitrocellulose or PVDF	10 V constant for 1 hour	Start: 65–85 mA

*Assemble the gel/membrane sandwich in reverse order so that the membrane is on the cathode side (–) of the gel (page 27).

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Using the XCell II™ Blot Module, Continued

Overnight Blotting

For overnight blotting, perform transfer in the cold room with low power to prevent overheating. Transfer at constant voltage of 10–15 V overnight. Depending on the transfer efficiency, adjust the transfer conditions accordingly.

Cleaning the Blot Module

Rinse the blot module with distilled 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. Do not submerge the blot module or soak overnight in nitric acid. Use gloves when preparing the nitric acid solution. Once the build-up is removed, rinse the module at least three times in deionized water.

Continued on next page

Post Transfer Analysis

After the transfer, 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 Invitrogen (page 32) 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 (page 32) 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 (page 32) 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 (page 32) 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 23. Refer to the manufacturer's recommendations for optimizing immunodetection.

Testing the Efficiency of Blotting

Introduction

Once you have performed the transfer, you may check the efficiency of transfer by using any one of the methods described below. Testing the blotting efficiency helps you evaluate the transfer and optimize transfer parameters to obtain an efficient transfer.

We recommend testing the efficiency of blotting when you are performing the western transfer for the first time and if you have changed the buffer system or gel type.

Materials Needed

- Pre-stained standards (page 32)
- Ponceau S or Coomassie® stain
- Transfer membrane
- Previously transferred mini-gel (maximum gel size 9 cm × 9 cm)

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Testing the Efficiency of Blotting, Continued

Procedure

The following methods can be used to test the efficiency of protein transfer.

Using pre-stained standards:

This is the most convenient way to monitor the efficiency of transfer. Pre-stained standards are protein markers that are covalently bound to a synthetic dye. This enables visualization of the protein markers during electrophoresis and after the transfer. Invitrogen offers three types of pre-stained standards (page 32 for ordering information). The transfer efficiency is good, if most of the standards have transferred to the membrane. Note that high molecular weight standards do not always transfer completely and this is not indicative of incomplete transfer.

If none of the standards or only a few have transferred to the membrane, you may have to optimize the transfer conditions. Refer to **Optimizing Blotting Parameters** on the next page and the **Troubleshooting** section on page 23.

Staining the gel:

The gel can be stained with Coomassie® Blue staining or a staining method of choice after the transfer to determine the transfer efficiency. If significant amount of proteins are still present on the gel after staining, this indicates poor transfer. You may need to optimize the transfer as described on the next page.

Staining the membrane:

Stain the membrane after the transfer to evaluate the transfer efficiency. If you are using the membrane for peptide sequencing, you may stain the membrane with Coomassie® Blue stain.

If you are using the membrane for immunodetection, you can stain the membrane with a temporary stain such as Ponceau S (see page 18 for Ponceau S staining). After visualizing the transferred protein bands on the membrane, you can rinse the membrane with deionized water to completely remove the staining or incubate the membrane directly in the blocking solution. Ponceau S stain does not interfere with most immunodetection methods.

Optimizing Blotting Parameters

Introduction

Each parameter of the blotting protocol plays a role during the transfer of proteins. An ideal blotting protocol balances each parameter to provide efficient transfer of proteins. When using the XCell II™ Blot module, most proteins will transfer efficiently using the protocol on page 12.

Based on specific properties of a protein or a set of proteins, some optimization of the blotting protocol may be necessary. Review the points described below to optimize the blotting protocol.

Gel Percentage

Choose the lowest percentage acrylamide appropriate for the molecular weight of your protein or proteins of interest. Gradient gels are excellent for blotting a range of protein sizes, as the porosity of the gel matrix is well matched with the different sizes of the proteins.

Some proteins can be equally well resolved on a Tricine gel or Tris-Glycine gel. In general, a Tricine gel will resolve the same range of proteins as a higher percentage Tris-Glycine gel and can be a better choice for some transfers.

Gel Thickness

The 1.0 mm thick gels are better for blotting. Be sure to scale your sample load appropriately for the sensitivity of your antibody detection method.

Field Strength

A higher field strength (volts/cm) may help larger proteins to transfer, but may also cause smaller proteins to pass through the membrane without binding. Our recommended condition for most proteins is 25 Volts for 90 minutes. You may make minor adjustments (± 5–10V) accordingly, when necessary. See **Transfer Conditions**. (page 16).

Alcohol in Transfer Buffer

Decreasing or eliminating alcohol may improve the transfer of some proteins, especially large proteins.

For blotting Novex® Tris-Glycine Gels and 2 NuPAGE® Novex® Gels, 20% methanol is added to the transfer buffer (page 8). This is balanced by the residual SDS in the gel from the running buffer. Keep this in mind when adjusting the methanol content in transfer buffer.

Continued on next page

Optimizing Blotting Parameters, Continued

Transfer Time

Increasing the transfer time to two hours will improve transfer of most proteins, but may cause the smaller proteins to pass through the membrane. Transfer time usually has little influence on the detection of proteins that remain bound to the membrane. Note that transfer times longer than 2 hours at the recommended power settings do not greatly improve transfer of proteins that have failed to transfer completely in 2 hours. This may be due to the exhaustion of the buffer or partial fixation of the protein in the gel as a result of the removal of SDS or a conformational change in the proteins during the transfer interval.

SDS in Transfer Buffer

Adding 0.01% to 0.02% SDS to the transfer buffer will facilitate the transfer of proteins, especially large proteins, but may reduce binding of proteins to the membrane, especially nitrocellulose membranes.

In the blotting protocol on page 12, the gel is not incubated in the transfer buffer, leaving residual SDS in the gel. This is balanced by the 20% methanol added to the transfer buffer. Keep this in mind when adjusting the SDS content in the transfer buffer.

Charge of Protein

For more basic proteins, use a carbonate buffer. The high pH of the buffer confers a higher negative charge on the more basic proteins and cause them to migrate faster. Carbonate buffers improve binding detection for some systems. Due to the high ionic strength of the carbonate buffer, excessive heat may be generated during blotting.

Hint

We recommend using two membranes in tandem during initial blotting to closely monitor the protein transfer and then perform the same visualization technique on both membranes. Monitor whether the primary membrane located next to the gel retains majority of the sample. If the sample is detected on the membrane placed closer to the anode (further away from the gel), reduce the rate of transfer by lowering the field strength, allowing more time for protein capture on the primary membrane. Adjust the blotting protocol accordingly using the guidelines included in this section.

Troubleshooting

Introduction

Review the information provided below to troubleshoot your experiments.

Problem	Cause	Solution
No proteins transferred to the membrane	Gel/membrane sandwich assembled in a reverse direction such that proteins have migrated out into the solution	Assemble the sandwich in the correct order using instructions provided on page 12.
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	• 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	• 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.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
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 8.
	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.
Power supply shuts off using recommended blotting conditions	High ionic strength of the transfer buffer	Prepare the buffer as described on page 8.
	Power supply is operating at a current close to the current limit of the power supply	Use a power supply with higher limits.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
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 gel/membrane 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.

Special Applications

Introduction

Additional protocols are provided below for certain special transfer applications such as blotting of IEF gels (next page), native gels (page 28), and semi-dry blotting (page 29) using the XCell II™ Blot Module. These protocols are slightly different than the standard blotting protocol described on page 12.

Materials Needed

- Previously electrophoresed mini-gels (maximum gel size 9 cm × 9 cm)
- 0.7% acetic acid in deionized water (for blotting IEF gels)
- Native transfer buffer for blotting native gels (25 mM Tris Base, 25 mM glycine, pH 9.2)

For semi-dry blotting

- Methanol
- Semi-dry transfer unit
- Filter papers
- NuPAGE® Transfer Buffer
- NuPAGE® Antioxidant

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Blotting IEF Gels

Novex® pre-cast IEF Gels are composed of 5% polyacrylamide and are more susceptible to hydrolysis due to the heat generated with the recommended blotting protocol. The following protocol is optimized to prevent hydrolysis and effective transfer of basic proteins due to the low pH of the transfer buffer.

1. Chill the 0.7% acetic acid, which will be used later for transfer.
2. After electrophoresis of the gel, equilibrate the gel in 0.7% acetic acid for 10 minutes.

   Tip: The 5% polyacrylamide gels are more sticky and difficult to handle than higher percentage polyacrylamide gels. To lift the gel from the equilibration solution, submerge the filter paper under the gel while the gel is floating in the equilibration solution. When the gel is in the correct position, lift up the filter paper to attach the gel to the filter paper. This prevents the gel from sticking to the filter paper before it is in the proper position and avoids handling of the gel.
3. Assemble the gel/membrane sandwich as described on page 12, except in a **reverse order** so that the membrane is on the cathode (-) side of the gel. This is the opposite of a typical western blotting protocol, where the negatively charged protein will migrate toward the anode (+) during the transfer.
4. Transfer for 1 hour at 10 V constant.

Continued on next page

Blotting Native Gels

During SDS-PAGE all proteins have a net negative charge due to the SDS in the sample buffer and the running buffer. Proteins separated during native gel electrophoresis do not have a net charge which may cause problems during the transfer. It is possible that some native proteins may have a higher pI than the pH of the Tris-Glycine Transfer Buffer used for standard western transfer protocols. Review the guidelines below to increase the transfer efficiency of native proteins.

- Increasing the pH of the transfer buffer to 9.2 (25 mM Tris Base, 25 mM glycine, pH 9.2), allows proteins with pI below 9.2 to transfer towards the anode electrode
- Place a membrane on both sides of the gel if you are using the regular Tris-Glycine Transfer Buffer, pH 8.3. If there are any proteins that are more basic than the pH of the transfer buffer, they will be captured on the membrane placed on the cathode side of the gel
- Incubate the gel in 0.1% SDS for 15 minutes before blotting with Tris-Glycine Transfer Buffer. The small amount of SDS will render enough charge to the proteins so they can move unidirectionally towards the anode and in most cases will not denature the protein

It is more likely for native proteins to diffuse out of the membrane into the solution during the blocking or antibody incubation steps, as the native proteins tend to be more soluble. We recommend fixing the proteins to the membrane to prevent diffusion of the proteins. The proteins can be fixed by air drying the membrane or incubating the membrane in 5–10% acetic acid for 15 minutes followed by rinsing the membrane with deionized water and then air drying.

By performing any of these two fixing methods the proteins will be sufficiently unfolded to expose hydrophobic sites and will bind more efficiently to the membrane.

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Semi-Dry Blotting of NuPAGE® Novex Bis-Tris Gels

The NuPAGE® Novex Bis-Tris Gels do not transfer efficiently using a semi-dry transfer cell as compared to blotting with XCell II™ Blot Module. If you decide to use semi-dry blotting for NuPAGE® Novex Bis-Tris Gels, use the protocol provided below to ensure efficient transfer.

1. Prepare 100 mL of 2X NuPAGE® Transfer Buffer from 20X NuPAGE® Transfer Buffer as follows:

NuPAGE® Transfer Buffer (20X)	10.0 mL
NuPAGE® Antioxidant (reduced sample)	0.1 mL
Methanol	10.0 mL
Deionized Water	79.9 mL
Total Volume	100 mL

   If you are blotting large proteins, see the **Note** below.

2. Soak the filter paper and transfer membrane in the transfer buffer.
   - If you are using Novex® pre-cut membrane/filter sandwiches, use three filter papers (0.4 mm/filter in thickness) on each side of the gel or membrane.
   - If you are not using the Novex® pre-cut membrane/filter sandwiches, use two thick filter papers.
3. Assemble the gel/membrane/filter paper sandwich on top of the anode plate as follows:

   Filter Paper
   Filter Paper
   Filter Paper
   Membrane
   Gel
   Filter Paper
   Filter Paper
   Filter Paper
4. Perform the transfer at 15 V constant for 15 minutes if you are using the Bio-Rad Trans-Blot Semi-Dry Cell. For any other semi-dry transfer cell, follow the manufacturer's recommendations.

   Note: For transfer of large proteins (>100 kDa), pre-equilibrate the gel in 2X NuPAGE® Transfer Buffer (without methanol) containing 0.02–0.04% SDS for 10 minutes before assembling the sandwich.

Buffer Recipes

20X NuPAGE® Transfer Buffer

20X NuPAGE® Transfer Buffer is available from Invitrogen (page 32).

1. To prepare 20X NuPAGE® 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 NuPAGE® 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.

25X Novex® Tris-Glycine Transfer Buffer

25X Novex® Tris-Glycine Transfer Buffer is available from Invitrogen (page 32).

1. To prepare 25X Novex® 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 the buffer at room temperature. The buffer is stable for 6 months at 25°C.
4. For transfer, dilute the 25X Novex® Tris-Glycine Transfer Buffer as described on page 8.

Continued on next page

Buffer Recipes, Continued

5X Novex® TBE Running Buffer

5X Novex® TBE Running Buffer is available from Invitrogen (see next page).

1. To prepare 5X Novex® TBE Running Buffer, dissolve the following reagents in 950 mL of deionized water:

		Concentration (1X)
Tris Base	54.0 g	89 mM
Boric Acid	27.5 g	89 mM
EDTA (free acid)	2.9 g	2 mM

2. Mix well and adjust the volume to 1,000 mL with deionized water. The pH of the buffer is 8.3.
3. Store the buffer at room temperature. The buffer is stable for 6 months at 25°C.
4. For transfer, dilute the 5X Novex® TBE Running Buffer as described on page 8.

Accessory Products

Additional Products

Ordering information for electrophoresis products available separately from Invitrogen is provided below. For detailed information, visit the www.invitrogen.com or call Technical Support (next page).

Product	Quantity	Catalog no.
NuPAGE® Novex 4–12% Bis-Tris Gel	10 gels/box	NP0321BOX
Novex® 10% Tris-Glycine Gel	10 gels/box	EC6075BOX
XCell *SureLock*™ Mini-Cell & XCell II™ Blot Module	1 unit	EI0002
Sponge Pad for blotting (8)	Each	EI9052
Gaskets (2)	Each	EI9016
PowerEase® 500 Power Supply	1 unit	EI8600
Novex® Sharp Pre-Stained Protein Standard	2 × 250 µL	LC5800
MagicMark™ XP Western Protein Standard	250 µL	LC5602
SimplyBlue™ SafeStain	1 L	LC6060
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
Nylon Membrane (0.45 µm pore size)	20 membrane/filter paper sandwiches	LC2003
WesternBreeze® Chromogenic Western Blot Detection Kit	1 Kit (Anti-Mouse)	WB7103
	1 Kit (Anti-Rabbit)	WB7105
WesternBreeze® Chemiluminescent Western Blot Detection Kit	1 Kit (Anti-Mouse)	WB7104
	1 Kit (Anti-Rabbit)	WB7106
Novex® Tris-Glycine Transfer Buffer (25X)	500 mL	LC3675
NuPAGE® Transfer Buffer (20X)	125 mL	NP0006
Novex® TBE Running Buffer (5X)	1 L	LC6675
NuPAGE® Antioxidant	15 mL	NP0005

Technical Support

World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog

Additional product information and special offers

Contact us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).

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SDS Requests

Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

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Technical Support, Continued

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