Table of Contents

1.0 Purpose 1
2.0 Scope 1
3.0 Authority and Responsibility 1
4.0 Materials and Equipment 1
5.0 Procedure 2
7.0 References and Related Documents 6
8.0 Change Summary 6

1.0 Purpose

This SOP describes how to electrophoretically transfer proteins from a gel to a suitable membrane for subsequent visualization

2.0 Scope

Biopharmaceutical Quality Control (BQC) personnel will perform this procedure. Other Biopharmaceutical Development Program (BDP) personnel may use this protocol for development or in-process analysis.

3.0 Authority and Responsibility

3.1 The Manager, Process Analytics/ Quality control (PA/QC) is responsible for assignment of this procedure and training of personnel.

3.2 PA/QC personnel are responsible for the performance of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Xcell II Mini-Cell and Blot Module (Catalog Number EI9001 and EI9051).

4.2 Previously electrophoresed mini-gels, see NOVEX catalog for selection of NOVEX Pre-Cast Mini Gels.

4.3 Pre-Cut Blotting Membranes: Nitrocellulose (Catalog Number LC2001, BDP NP 31062), PVDF (Catalog Number LC2002, BDP PN 31060) or other approved membrane.

4.4 Blotting pads (Catalog Number EI 9052, BDP PN 21634).

4.5 Methanol (BDP PN 10115).

4.6 Sartorius Water Purification System.

4.7 Novex Tris-Glycine Transfer Buffer (25X) (BDP PN 30042, Catalog Number LC3675 or equivalent), or Novex NuPAGE Transfer Buffer (20X) (BDP PN 30079, Catalog Number NP0006 or equivalent).

4.8 NuPAGE Sample Antioxidant (BDP PN 30076, Catalog Number NP0005).

NOTE: For Reduced Samples Only.

4.9 Shallow tray for equilibration of membranes, filter paper, and blotting pads.

4.10 Novex Power Ease 500 Power Supply (Catalog Number EI8600 or equivalent).

4.11 Graduated Cylinders

5.0 Procedure

NOTE: During the following procedures, gloves must be worn at all times. This prevents the contamination of gels and membranes and also prevents the researcher from contact with irritating salts and chemicals commonly used in electrophoresis and electro transfer.

5.1 Preparation of Blotting

5.1.1 Prepare the 1x Transfer Buffer.

If using Novex Tris-Glycine Transfer Buffer, pour 40 mL of 25x transfer buffer 200 mL Methanol, and 760 mL of deionized water into a graduated cylinder when making up 1L. Mix gently. If using NuPAGE Transfer Buffer and the samples are non-reduced, pour 50 mL of (20x) Transfer Buffer, 100 mL Methanol and 850 mL deionized water into a graduated cylinder. Mix gently. If the samples are reduced, add 1 mL NuPAGE Sample Antioxidant to the 1 L NuPAGE Transfer Buffer (1x).

NOTE: When making up reagents, record steps in BQC Solution Logbook. Label the solution with the name, prepared by, date prepared, expiration date, and the BQC number. Store the solution at room temperature.

5.1.2 Prepare the transfer membrane and filter paper.

Cut the selected transfer membrane and filter paper to the dimensions of the gel, or use NOVEX precut membrane/filter paper sandwich assemblies (Catalog Number LC2001, BDP PN 21633, LC2002, BDP PN 20370, or LC2003 for nitrocellulose, PVDF or Nylon, respectively).

a) Using a VWR marker, place a small mark in the left-top corner of the membrane. Pre-wet the PVDF membrane in methanol, and then place in a shallow dish of transfer buffer for several minutes.

b) If using nitrocellulose or Nylon membrane, place the membrane directly into transfer buffer.

c) Soak the two sheets of filter paper briefly in transfer buffer immediately prior to use.

5.1.3 Prepare the polyacrylamide gel.

NOTE: The gel is run according to ***SOP 22101***, ***SDS-PAGE Electrophoresis Using Invitrogen Tris-Glycine Gels*** or ***SOP 22176, SDS Page Gel Electrophoresis Using the NuPAGE/Bis-Tris gels***.

a) If using the Tris-Glycine transfer buffer, prepare the gel by soaking it in 50-100 mL of 1x transfer buffer for 5-10 minutes while shaking gently. This step removes excess buffer salts and detergents, which may increase the conductivity of the transfer buffer and result in increased transfer temperatures.

b) If using the NuPAGE transfer buffer, use the gel immediately following the run. Do not soak the gel in transfer buffer.

5.1.4 Prepare the blotting pads.

Soak the blotting pads in 1x Transfer Buffer until saturated. Remove the air pockets and bubbles by squeezing the pads while they are submerged in buffer. Removing the air bubbles is essential, as they can block the transfer of biomolecules if they are not removed.

5.2 Xcell II Blot Module Assembly

NOTE: Position the gel/membrane sandwich so that the gel will be centered on the electrode plates when the blot module is upright in the Xcell II Mini-Cell unit. This will ensure that the entire gel sandwich is immersed in the transfer buffer.

5.2.1 Follow step 5.2.5 when transferring one gel. Follow step 5.2.6 when transferring two gels.

a) Place 1-2 saturated blotting pads on the electrode of the Cathode (-) Core. The Cathode (-) is deeper of the two cores and the corresponding electrode plate is a darker shade of gray.

b) Place one of the prepared filter papers on top of the blotting pads.

NOTE: The surface must be thoroughly saturated with transfer buffer, and all trapped air bubbles between blotting pads and filter paper must be removed by using western blot roller or gently pressing on the filter paper.

5.2.2 Transfer the polyacrylamide gel from the transfer buffer equilibration step and place it on top of the filter paper. Place one corner of the gel on a corner of the filter paper and gently lay the gel down so that no bubbles are trapped between the gel and the filter paper. **The gel should be placed facedown.**

5.2.3 Place the prepared blotting membrane on top of the gel face down. The mark in the left-top corner must appear to be on the right corner when the membrane is face down. Start at the one edge and carefully lay the membrane down so that no bubbles are trapped between the gel and the membrane.

NOTE: The PVDF membrane must be wet with methanol before placing the membrane on top of the gel.

5.2.4 Place the remaining piece of prepared filter paper on top of the membrane. Again, remove the trapped air bubbles by gently pressing on the filter paper.

5.2.5 When transferring one gel, place two blotting pads on top of the filter paper and proceed to step 3.2.6. Refer to the following illustration for proper sandwich assembly.

Gel/Membrane Sandwich – 1 Gel

Anode +

Blotting Pad

Blotting Pad

Filter Paper

Transfer Membrane

Gel

Filter Paper

Blotting Pad

Blotting Pad

Cathode –

5.2.6 When transferring two gels, place one blotting pad on top of the filter paper and start to build a second sandwich. Refer to the following illustration for proper sandwich assembly.

Gel/Membrane Sandwich – 2 Gels

Anode +

Blotting Pad

Blotting Pad

Filter Paper

Transfer Membrane

2nd Gel

Filter Paper

Blotting Pad

Filter Paper

Transfer Membrane

1st Gel

Filter Paper

Blotting Pad

Cathode –

5.3 Place a Anode (+) Core on top of the gel/membrane sandwich(es). The sandwich(es) must be held securely between the two halves of the blot module (i.e., Cathode (-) Core and Anode (+) Core) ensuring complete contact of all components. Because blotting pads tend to lose resiliency after many uses, an additional pad may be required to ensure a snug fit between both sides of the blot module. Pads must be replaced when they discolor and begin to lose resiliency.

5.4 Hold the blot module together at the top and slide it into the guide rails on the lower buffer chamber. The blot module will only fit into the lower buffer chamber one way, so that the plus sign can be seen in the upper left-hand corner of the chamber. Properly placed, the inverted gold post on the right-hand side of the blot module will fit into the hole next to the upright gold post on the right side of the lower buffer chamber.

5.5 Place the wedge so that the flat part is against the blot module. Using the off-white handle lock the wedge into place.

5.6 Fill the blot module with transfer buffer until the gel/membrane sandwich is covered in transfer buffer. The transfer buffer level must be just below the white screws on the cathode core.

5.7 Fill the lower buffer chamber by pouring deionized water or transfer buffer in the gap between the front of the blot module and the front of the lower buffer chamber. The water level must be approximately 2 cm from the top of the lower buffer chamber.

5.8 Place the lid on top of the unit.

5.9 With the power turned off, plug the red and black leads into the power supply.

5.10 Press the power button and then start on the power supply. Choose Western Blot or NuPAGE Blot from the menu by pressing the arrow keys. Press the select button and by using the up arrow key, choose the number of Blot units to be run. Press select again and start to begin the Western Blot.

NOTE: The Western Blot runs for 1½ hours at 25V, 125mA and 17W. The NUPAGE Blot settings are 1 hour at 25V, 160mA and 17W.

5.11 Once the run is complete, turn off the power supply, disassemble the apparatus and remove the transfer membrane. At this point the membrane can be stained for detection of proteins or used for immunological detection of specific proteins (***SOP 22103, Immunodetection of Proteins Electroblotted to Membranes***).

6.0 Documentation

6.1 Record results on the BQC Test Request Form (Form 22002-01).

6.2 Results are given to the BQC Supervisor for review and signature. The results then go to BQA for review and signature. The original document is stored in the BQA Document Control Room.

7.0 References and Related Documents

Novex Precast Gel Instructions, or NuPAGE Electrophoresis System Bis/Tris Gels Instruction.

SOP 22002 *Request For Quality Control Testing*

SOP 22702 *Solutions Used in BQC*

SOP 22101 *SDS-PAGE Electrophoresis Using Invitrogen Tris-Glycine Gels*

SOP 22176 *SDS Page Gel Electrophoresis Using the NuPAGE/Bis-Tris gels.*

SOP 22103 *Immunodetection of Proteins Electroblotted to Membranes*

Form 22002-01 *BQC Test Request Form*

8.0 Change Summary