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Title: Use and Maintenance of the Invitrogen XCell II™ Blot Module

Version: v2

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SOP History		
Number	Date	Reason for Change
v1	01/02/2013	Original
V2	01/02/2018	Update and amend author detail

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use of the Invitrogen XCell II™ Blot Module in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to routine use and maintenance of the Invitrogen XCell II™ Blot Module within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Invitrogen XCell II™ Blot Module within the SASoM to comply with this SOP.

4.0 Procedure –

Principles of Operation:

The XCell II™ Blot Module allows a semi-wet transfer of proteins, which have been separated on a gel, to be transferred to a membrane which in turn can then be used to probe for a variety of proteins by antibodies.

Preparing transfer buffer, blotting pads, filter paper, and transfer membrane:

- Prepare transfer buffer as per manufacturer's instructions or based on transfer buffer recipes.

- Use transfer buffer to soak the blotting pads until saturated, removing air bubbles during the process.
- Soak filter paper in transfer buffer immediately before using.
- Prepare PVDF with a 30 second soak in Ethanol, then rinsing in deionised water before placing in transfer buffer, OR prepare nylon/nitrocellulose by placing directly in the transfer buffer.

Removing the gel and assembling the transfer apparatus:

- After electrophoresis, remove the gel cassettes from the mini-cell, handling by their edges only.
- Lay the gel cassettes (well side up) on a flat surface and carefully insert the Gel Knife's bevelled edge into the narrow gap between the two plates of the cassette.
- Push up and down on the Knife's handle to separate the plates until you hear a cracking sound (which means the bonds have been broken holding the plates together). Repeat until the two plates are completely separated.
- Upon opening the cassette, the gel may adhere to either side. Remove and discard the plate without the gel – proceed to blotting or staining.
- Remove wells on the gel with the gel knife.
- Place pre-soaked filter paper on top of the gel, and lay just above the 'foot' at the bottom of the gel (leaving the 'foot' of the gel uncovered. Remove trapped air bubbles by gently rolling over the surface with a pipette.
- Turn the plate over so the gel and filter paper are facing downwards over a gloved hand or a flat surface covered with Parafilm.
- Remove the gel (if on slotted plate) by using the gel knife to push the foot out of the slot in the plate and the gel will fall out easily. If 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. When gel is on a flat surface, cut the 'foot' off the gel with the gel knife.

Transferring one gel:

- Wet the surface of the gel with transfer buffer and place pre-soaked transfer membrane on the gel. Remove air bubbles by rolling a pipette over the surface
- Place pre-soaked filter paper on top of the transfer membrane, removing air bubbles.
- Place 2 soaked blotting pads in the cathode (-) core of the blot module (this is the deeper of the two cores with an electrode which is darker gray. Carefully pick up the gel assembly and place on the pad in the same sequence such that the gel is closest to the cathode plate.
- Add enough pre-soaked blotting pads to rise 0.5cm 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 two halves of the blot module ensuring complete contact of all components.
- 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 ~1cm at the top of the electrodes when the pads and assembly are in place.

- Hold the blot module together and slide it into the guide rails of the lower buffer chamber. The blot module fits in 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.
- 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.
- Fill the blot module with transfer buffer until the gel/membrane sandwich is covered in transfer buffer. (don't fill to the top as this will generate excess heat)
- Fill the outer chamber with ~650ml of deionised water by pouring in the gap between the front of the blot module and the front of the lower buffer chamber. The water level should reach about 2cm from the top of the lower buffer chamber.
- Place the lid on top of the unit.
- With the power OFF, plug the red and black leads into the power supply.
- Turn on the power supply and run transfer.

Transferring two gels:

- Wet the surface of the gel with transfer buffer and place pre-soaked transfer membrane on the gel. Remove air bubbles by rolling a pipette over the surface
- For both gels individually, place pre-soaked filter paper on top of the transfer membrane, removing air bubbles.
- Place 2 soaked blotting pads in the cathode (-) core of the blot module (this is the deeper of the two cores with an electrode which is darker gray. Carefully pick up the first gel assembly and place on the pad in the same sequence such that the gel is closest to the cathode plate.
- Add another pre-soaked blotting pad on top of the first membrane assembly.
- Position the second gel/membrane sandwich on top of the blotting pad in the correct orientation so that the gel is closest to the cathode side.
- Proceed as described for transferring one gel (above):
- Add enough pre-soaked blotting pads to rise 0.5cm 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 two halves of the blot module ensuring complete contact of all components.
- 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 ~1cm at the top of the electrodes when the pads and assembly are in place.
- Hold the blot module together and slide it into the guide rails of the lower buffer chamber. The blot module fits in 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.
- 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.
- Fill the blot module with transfer buffer until the gel/membrane sandwich is covered in transfer buffer. (don't fill to the top as this will generate excess heat)

- Fill the outer chamber with ~650ml of deionised water by pouring in the gap between the front of the blot module and the front of the lower buffer chamber. The water level should reach about 2cm from the top of the lower buffer chamber.
- Place the lid on top of the unit.
- With the power OFF, plug the red and black leads into the power supply.
- Turn on the power supply and run transfer.

5.0 Personal protection –
Howie coat must be worn at all times.

Gloves as specified in the appropriate COSHH RA

6.0 Spillages -
Always clean up any spills to both the Invitrogen XCell II™ Blot Module and the bench immediately after use.

Only you know what you have spilt and are aware of that chemicals hazard.

Mop up spills with paper towels. Wash the site of spillage with water & detergent.

7.0 General maintenance -
Clean surfaces of the apparatus with soft cloth and mild detergent.

8.0 Training –
All users have to be trained before using the Instrument by a designated person.

9.0 Related documents –

9.1 Equipment manual

9.2 Equipment Maintenance Information sheet

9.3 Risk assessments – RA/GEN/037

9.4 SOP SASoM/EQUIP/032
Use and Maintenance of the Biorad ‘200’, ‘300’, ‘1000’ and ‘3000’ Power-Packs

10.0 Approval and sign off –

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