Document Number:	**SASoM/METHOD/033.v6**
Title:	**Western Blot Polyacrylamide Gel Electrophoresis**
Version:	**V6**
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SOP History		
Number	Date	Reason for Change
V1	01/02/2013	Original
V2	01/02/2015	Update
V3	01/02/2017	Update
V4	08/03/2017	Amendments
V5	08/03/2019	Update
V6	08/03/2021	Update

1.0 Purpose –

This SOP describes the current procedure for Western Blot Polyacrylamide Gel Electrophoresis in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff in the SASoM involved with Western Blot Polyacrylamide Gel Electrophoresis.

3.0 Responsibilities –

All staff involved in with Polyacrylamide Gel Electrophoresis are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

Assemble and run the gel apparatus as in SASoM/EQUIP/008.

Note that you must have 2 gels in each tank so add a dummy gel if required.

Protein samples should have been normalised and prepared in an appropriate sample loading buffer.

Make up the resolving gel and stacking gel solutions as shown in the table below for the appropriate percentage gel, but do not add TEMED or AMPS until ready to pour as the gel will begin to set once these are added.

Add TEMED and AMPS to the resolving gel solution and mix (avoid bubbles) and pour gel (use a pastette if necessary), holding end of tube against the large plate (usually approx 5ml of resolving gel mix).

Add a little Isopropanol (propan-2-ol) or water to top of resolving gel using a fine-tipped pastette. Leave resolving gel to set (keep a small amount in a tube – when this has set the gel will be set).

Once the resolving gel has set (approx 20-30mins) wash layer of Isopropanol off with distilled water, drain water of onto tissue.

Drain off excess dH_20. Tilt the gel to one side and use blotting paper to remove the excess liquid.

Add TEMED and APS to stacking gel solution and mix gently.

Pour the stacking gel on top of the resolving gel. Immediately insert comb. Make sure there are no bubbles under/beside comb or samples will not move through the gel evenly. Leave stacking gel to set (keep a small amount in a tube – when this has set the gel will be set).

Once the stacking gel has set (approx 20 min), remove comb and immediately wash the wells with distilled water or running buffer using a fine-tipped pastette and ensure all bubbles are removed from the wells.

Remove plates from casting stand and assemble into running tank.

Add running buffer between the gels and allow to overflow into the outer section of the tank up to the shelf (approx 4-5cm).

These gel recipes are sufficient for 4 mini-gels or 2 large gels.

Resolving gel:

	5%	7.5%	10%	12%	15%	20%
Acrylamide (mL)	6.7	10.0	13.5	16.0	20.0	26.5
1M Tris, pH8.85 (mL)	15.0	15.0	15.0	15.0	15.0	15.0
10% SDS (mL)	0.4	0.4	0.4	0.4	0.4	0.4
dH_2O (mL)	17.9	14.6	11.1	8.6	4.6	0.0
TEMED (mL)	0.1	0.1	0.1	0.1	0.1	0.1
10% AMPS (mL)	0.1	0.1	0.1	0.1	0.1	0.1

3.6% Stacking Gel:

Acrylamide	3.6mL
0.375M Tris, pH6.8	10 mL
10% SDS	0.3mL
dH_2O	16 mL
TEMED	0.1mL
10% APS	0.1mL

Running Buffer:

Tris Base	3.03g
Glycine	14.42g
10% SDS	10ml
Distilled Water	up to 1l

Thaw samples, and then centrifuge to collect liquid at the bottom of the tubes. Heat samples at 95°C for 2-3min to denature proteins (can be done for 60min at 60°C if preferred, but ensure that you centrifuge afterwards to collect evaporated liquid). Centrifuge again.

In the fume cupboard if necessary (since the sample loading buffer contains β-mercaptoethanol), load samples ASAP - when samples cool refolding of protein may occur. Use gel loading tips if required. Load 5-10µl of marker to lane 1. Load up to 40µl per lane for each protein sample.

Run gel at 80V ~15min until the samples have left the wells and reached the border between the stacking and resolving gels. Turn up the voltage to 150V for ~45min. Run until dye front is close to bottom of the gel.

Preparation of the Immobilon®-P PVDF Membrane

PVDF membranes have high protein adsorption making for a more efficient transfer. The open pore structure makes it easy to access bound proteins and remove unbound probes. Advantages of PVDF membranes include (i) they won't crack or curl, and they can be cut without fracturing (ii) low background, (iii) broad solvent compatibility (iv) superior staining capabilities, (v) can be reprobed multiple times, and (vi) compatible with a variety of detection chemistries, including radioactive, chromogenic, chemiluminescent, fluorescent, and chemifluorescent techniques.

Cut a piece of the Immobilon-P membrane to the dimensions of the gel (13cm x 16cm for large Biorad Gels). Notch or label (in pencil) one corner of the membrane to correspond to a corner of the gel.

Soak the membrane in 100% methanol for 15 seconds. (Recycle the methanol into a dark bottle and use next time).

Transfer the membrane to a container of distilled water for 2 minutes – the membrane is hydrophobic so you may need to gently push it below the surface of the

water with flat-nosed forceps. CAUTION: Use care when handling the membrane to prevent tearing. Do not leave any dry spots that can inhibit the transfer.

Equilibrate the membrane for at least 5 minutes in Transfer Buffer prior to performing the transfer.

Transfer of the gel to the membrane.
Remove gel plate section from the electrophoresis tank.

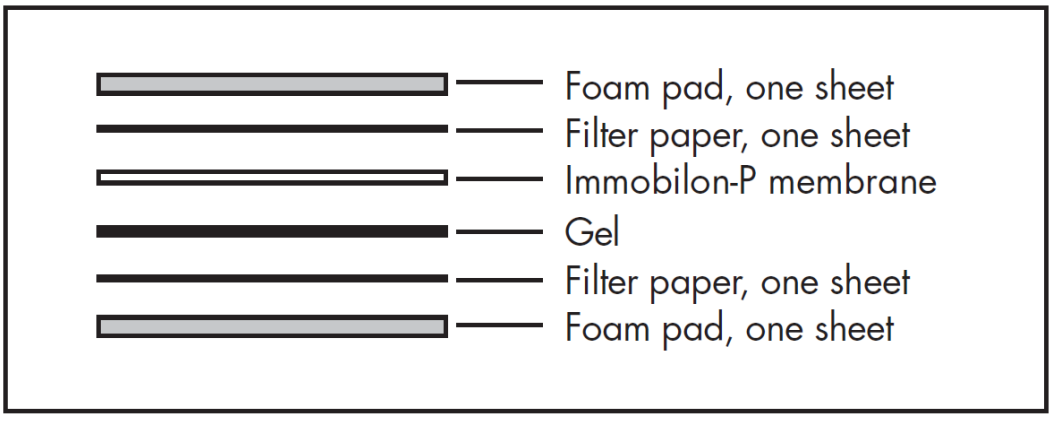
Carefully separate the glass plates using the black spacers. The gel should only stick to one plate, lay this flat and note corner corresponding to bottom corner closest to 1st sample loaded. Trim away the stacking gel and discard into a red 'chemical waste' bin. DO NOT let the gel dry out.

Place the cassette holder on the bench with the black side downwards. Place a foam pad on the black side of the cassette holder. Then place two sheets of filter paper on top of the pad. CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a pipette over the surface of each layer in the stack. Applying excessive pressure may damage the membrane and gel.

Place the gel on top of the filter paper. Then put the the Immobilon-P membrane on top of the gel.

Place two more sheets of filter paper on top of the membrane and then put a second foam pad on top of the filter paper.

The stack should look like this:



Close the tank transfer cassette holder.

Place the transfer cassette holder(s) in the tank blotting apparatus so that the black side of the cassette holder with the gel is facing the black cathode (-) and the clear side of the cassette holder is nearest the anode (+).

Add enough transfer buffer to the blotting apparatus to cover the cassette holder (approximately 1cm from the top). Place a magnetic stirring bar inside the tank and sit on a stirring plate.

Insert the black cathode lead (-) into the cathode jack. Insert the red anode lead (+) into the anode jack. Then connect the anode lead and cathode lead to their corresponding power outputs.

Turn on the power and run at 20-30V overnight in the coldroom.

Disassemble the tank the following day to release the membrane. The membrane should clearly show any pre-stained markers which were loaded on the gel.

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

Gloves as specified in the appropriate COSHH RA.

6.0 Spillages –
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should under go training in this technique before performing procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/013

8.2 SASoM/EQUIP/008
Use and Maintenance of the Mini-PROTEAN® II Electrophoresis apparatus

8.3 SASoM/METHOD/034
Western Blot Antibody Detection Using Licor Odyssey Scanner

9.0 Approval and sign off –

Author:		
Name:	Peter Mullen	
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Signature:		Date: 22/03/2021
Management Approval:		
Name:	Peter Mullen	
Position:	Research Fellow	
Signature:		Date: 22/03/2021
QA release by:		
Name:	Alex MacLellan	
Position:	QA Manager	
Signature:		Date: 22/03/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE