Document Number: **SASoM/METHOD/050.v5**

Title: **Biorad mini western blotting**

Version: **v5**

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Effective from:	09/09/2021
Valid to:	09/09/2023

SOP History		
Number	Date	Reason for Change
v1	29/08/2015	Original
v2	10/08/2015	Update
v3	10/08/2017	Update
V4	10/08/2019	Update
V5	09/09/2021	Update

1.0 Purpose –
This SOP describes the current procedure for Biorad mini western blotting for use in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in Biorad mini western blotting work.

3.0 Responsibilities –
All staff involved in cell culture 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 –

Before Starting:

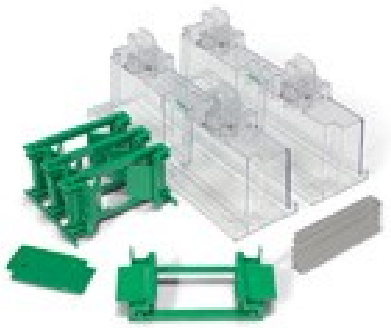
- Prepare 1mm plates (the ones with numbers) and glass plates (the ones without numbers). A group of plate + glass plate per gel.
- Casting clamp assembly

1x running buffer is: 25 mM Tris base, 190 mM glycine, 0.1% Glycine, 0.1% SDS, pH 8.3.
1x transfer buffer is: 25 mM Tris Base, 190 mM glycine, 0.1% SDS

A. Separating gel

1. Rinse the plates with ethanol under the sink and allow it to dry.

2. Prepare apparatus – Casting clamp assembly



- Plate with the numbers on the back
- Plate without numbers – glass plates - on the front
- Place it into the casting stand - green structure in the picture.

3. Test with water if it is leaking. If it is, rearrange the plates and try again. If it is not, proceed.

4. Separating Gel recipe

10% Tris/ Glycine polyacrylamide Gel					
	5mL	**10mL**	**15mL**	**20mL**	**50mL**
Water	2.45	4.9	7.35	9.8	24.5
30% AA/Bis	1.65	3.3	4.95	6.6	16.5
2M tris-HCl **pH 8.8**	0.9	1.8	2.7	3.6	9
10% APS **(µL)**	50	100	150	200	500
TEMED **(µL)**	5	10	15	20	50

- Water should be MiliQ water
- 30% AA/Bis kept in the fridge
- 2M tris-HCl **pH 8.8** kept at room temperature. ATTENTION to the pH value!!!
- 10% APS kept in the fridge
- TEMED is kept in the fridge and should be added under the fume hood.

4.1. Prepare the recipe using a 15mL or a 50mL tube, as appropriate.

4.2. Using a P1000 add the solution to the plates, 1 mL at the time, up to "one nail" below the green line (approximately 4 mL per gel).

4.3. Add 100% butanol for the interface layer.

4.4. Allow gel to polymerize. Wait 20 minutes.

B. Stacking gel

1. Stacking Gel recipe

10% Tris/ Glycine polyacrylamide Gel					
	5mL	**10mL**	**15mL**	**20mL**	**50mL**
Water	3.55	7.1	10.65	14.2	35.5
30% AA/Bis	0.825	1.65	2.475	3.3	8.25
1M tris-HCl **pH 6.8**	0.625	1.25	1.875	2.5	6.25
10% APS **(µL)**	50	100	150	200	500
TEMED **(µL)**	5	10	15	20	50

- Water should be MiliQ water
- 30% AA/Bis kept in the fridge
- 1M tris-HCl **pH 6.8** kept at room temperature. ATTENTION to the pH value!!!
- 10% APS kept in the fridge
- TEMED is kept in the fridge and should be added under the fume hood.

2. Remove butanol layer rinse off into the sink.

3. Using a P1000 add the solution to the plates, approximately 1 mL per gel, up to above the second green line.

4. Insert 10 wells comb (the letters to the front). CAREFUL when inserting the comb, especially with eyes as the solution is toxic. Clean, with ethanol, the liquid that drops out.

   Here, if it is late, the gel can be kept at the cold room (4ºC) until the next day.

5. Allow gel to polymerize. Wait 15 minutes.

C. Samples Preparation and gel Electrophoresis

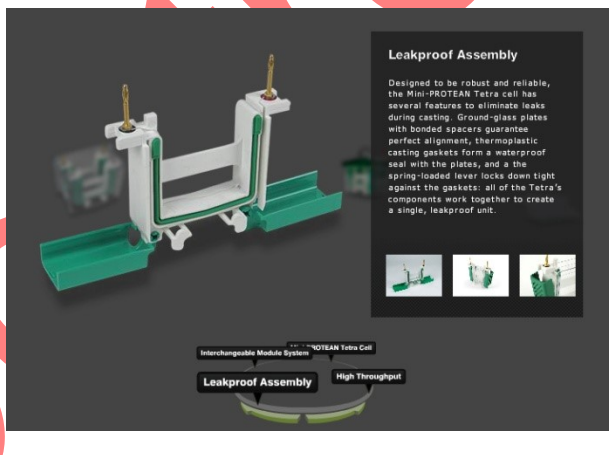
- Before starting loading the samples, make sure that all samples were previously quantified ensuring that the same concentration of samples is being loaded (30µg in 20 µL). This protein quantification is obtained using BCA Protein Assay Kit.

1. In eppendorf tubes, prepare samples adding:

   - MiliQ Water
   - 6x dye (3.3 µl)
   - Protein (quick spin after thawing)
   - Marker - 5 µL 6x dye + 5 µL Marker (Amersham, Full-Range Rainbow, Molecular Weight Markers, GE Healthcare).

2. Turn on the heating block to 100ºC.

3. Prepare Electrophoresis Chamber. Remove the gel from the casting clamp assembly and put it into casting gaskets (see figure).



4. Add 1x Running Buffer. This buffer can only be used once hence, need to make fresh each time.

5. Setup the gel into the chamber. Make sure the gel is turned to the right side, which is the chamber inside.

6. Add 1x running buffer to the chamber inside up to the top. Just here remove the comb, making sure there are no bubbles into the wells.

7. Pre-boiled samples for 5 minutes. Then spin down before loading. Pay attention to the side from where you are starting. Draw a scheme of the loading samples sequence.

8. Run gel for approximately 1h 30min using a voltage of 85V, until the dye is out of the gel.

D. Transfer Step – proteins from the gel to the membrane

1. Transfer buffer can be re-used 3 times. It is kept at 4ºC, cold room. However, after 3xs new buffer should be prepared following the recipe and **by this order**:
    a) 20% Transfer buffer 5x (200 mL of 5xTransfer buffer)
    b) dWater (600 mL)
    c) 20% of 100% methanol (200 mL 100% methanol).

2. The running buffer can be disposed in the sink.

3. Prepare per gel

    - one piece of membrane
    - 4 pieces of Whatman paper
    - 2 sponges

- Activate membrane soaking it in 100% methanol. Leave it for 2 to 3 minutes. Quickly rinse it in distillated water and then soak it into transfer buffer.
- Soak both the whatman paper and the sponges in transfer buffer.
- Add transfer buffer to the tank to about half full
- **Attention** – to remove the gel rinse the plates with water. Remove the gels avoiding gel to break. Remove the wells with a spatula.

4. Prepare the gel-holder cassette

5. Order of the sandwich:

   - Black side of the cassette
   - One sponge
   - 2 whatman papers (also called blotting paper)
   - **GEL – Lane 1 of the gel should go on the right side of the membrane**
   - Activated membrane – be sure that there are not bubbles. Can use a pipette to avoid it, however, use extremely carefully/ slowly.
   - 2 whatman papers
   - One sponge
   - White side of the cassette

6. Put the electrode assembly into the tank.

7. Firmly close the gel-holder cassette and put it into the electrode assembly in the right order (black-black/ white/red) – see picture - in the tank with transfer buffer. Air bubbles will form unless cassette pressure is released in liquid.

8. Introduce the ice pack into the apparatus in order to reduce protein degradation by temperature.

9. If necessary, add more 1x transfer buffer to the chamber to reach the "Blotting" signal.

10. Run at 225 Amp, approximately 80V for 1h15 minutes.

E. Blocking Step and Odyssey LiCor detection.

Follow protocol for antibody detection using the LiCor Odyssey scanner.

5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

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 100% ethanol or water.

7.0 Training -

All staff should be trained in sterile TC techniques before starting any TC work

8.0 Related documents –

8.1 Risk assessment
CHARM-RA22140-SDS-Page western Blotting

8.2 SOPs
SASoM/EQUIP/033 - Mini Transblot Apparatus
SASoM-METHOD-034-Western Blot Antibody Detection Using Licor Odyssey Scanner
SASoM/METHOD/033
Western Blot Polyacrylamide Gel Electrophoresis

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 09/09/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 09/09/2021

QA release by:

Name: John O' Connor

Position: QA Manager

Signature:  Date: 09/09/2021

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