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Title: **Use and Maintenance of the Mini-PROTEAN® II Electrophoresis apparatus**

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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
V2	01/01/2018	Update

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use and maintenance of the Mini-PROTEAN® II gel electrophoresis apparatus in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to routine use and maintenance of the Mini-PROTEAN® II within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Mini-PROTEAN® II within the SASoM to comply with this SOP.

4.0 Procedure –

Principles of Operation:

Electrophoresis gel tanks allow the rapid analysis of protein samples in miniature polyacrylamide gels.

Assembling the Glass Plate Sandwiches:

Assemble the gel sandwich on a clean surface.

Lay the longer rectangular glass plate down first, then place two spacers of equal thickness along the short edges of the rectangular plate.

Next, place the shorter glass plate on top of the spacers so that the bottom ends of the spacers and glass plates are aligned.

At this point, the spacers should be sticking up about 5 mm above the long glass plate.

Loosen the four screws on the clamp assembly and stand it up so that the screws are facing away from you. Firmly grasp the glass plate sandwich with the longer plate facing away from you, and gently slide it into the clamp assembly along the front face of the acrylic pressure plate. The longer glass plate should be against the acrylic pressure plate of the clamp assembly. Tighten the top two screws of the clamp assembly.

Place the clamp assembly into the alignment slot so that the clamp screws face away from you. Loosen the top two screws to allow the plates and spacers to settle against the casting stand base. Insert the Mini-PROTEAN II alignment card between the glass plates, in order to position the spacers properly. Gently tighten both pairs of screws.

Remove the alignment card. Pull the completed sandwich from the alignment slot. Check that the plates and spacers are flush at the bottom. If not, realign the sandwich as above.

Using the levelling bubble, level the casting stand with the alignment slot facing you.
Check to see that the removable gray silicone gaskets are clean and free of residual acrylamide to insure a good seal. Place the silicone rubber gaskets on top of the red foam pads of the casting stand slots

Transfer the clamp assembly to one of the casting slots in the casting stand. If two gels are to be cast, place the clamp assembly on the side opposite the alignment slot to make aligning the next sandwich easier.

Attach the sandwich in the following way: Put the acrylic pressure plate against the wall of the casting slot at the bottom, so the glass plates rest on the rubber gasket. Snap the acrylic plate underneath the overhang of the casting slot by pushing with the white portions of the clamps. Do not push against the glass plates or spacers. This could break the plate.

If another gel is to be cast, align the sandwich plates in the clamp assembly, and then transfer to the other casting slot of the casting stand. The gel sandwiches are now ready for casting.

Casting Gels:
Place a comb completely into the assembled gel sandwich. With a marker pen, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

Prepare separating gel and pour the solution to the pen mark.

Immediately overlay the monomer solution with water.

Allow the gel to polymerize for 45 minutes to 1 hour.
Remove water by draining

Place a comb in the gel sandwich and tilt it so that the teeth are at a slight (~10°) angle. This will prevent air from being trapped under the comb teeth while the monomer solutions are poured.

Prepare the stacking gel monomer solution

Pour until all the teeth have been covered by solution. Then properly align the comb in the sandwich and add monomer to fill completely.

Allow the gel to polymerize 30-45 minutes. Remove the comb by pulling it straight up slowly and gently.

Rinse the wells completely with distilled water or running buffer. The gels are now ready to be attached to the inner cooling core, the sample loaded, and the gels run.

Assembly:
Release the clamp assemblies/gel sandwiches from the casting stand.

Lay the inner cooling core down flat on a lab bench. With the glass plates of the gel sandwich facing the cooling core (and the clamp screws facing out), carefully slide the clamp assembly wedges underneath the locator slots on the inner cooling core until the inner glass plate of the gel sandwich butts up against the notch in the U-shaped gasket.

While pushing the clamp assembly slightly up toward the top of the locator slots, snap the clamp assembly fully onto the cooling core by pressing at the bottom of the clamp assembly until the cooling core latch engages each side of the clamp assembly. (Do not pull out on cooling core latch at the same time.)

Turn over the inner cooling core and attach another clamp assembly to the other side of the core in the same manner.

Loading Sample Wells:
Lower the inner cooling core into the lower buffer chamber of the Mini-PROTEAN II cell.

Add approximately 115 ml of buffer to the upper buffer chamber. Fill until the buffer reaches a level halfway between the short and long plates. Do not overfill the upper buffer chamber.

Pour the remainder of the buffer into the lower buffer chamber so that at least the bottom 1 cm of the gel is covered. Remove any air bubbles from the bottom of the gel so that good electrical contact is achieved. This can be done by swirling the lower buffer with a pipette until the bubbles clear.

Load the samples into the wells under the electrode buffer with a Hamilton syringe or with a pipette.

Running the Gel:
Place the lid on top of the lower buffer chamber to fully enclose the cell. The correct orientation is made by matching the colours of the plugs on the lid with the jacks on the inner cooling core.

Attach the electrical leads to a suitable power supply (200 V minimum) with the proper polarity.

Apply power to the Mini-PROTEAN II cell and begin electrophoresis, the usual run time is approximately 45 minutes.

After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.

Remove the cell lid and carefully pull the inner cooling core out of the lower chamber. Pour off the upper buffer.

Lay the inner cooling core on its side and remove the clamp assembly by pushing down on both sides of the cooling core latch and up on the clamps until the clamp assembly is released. Slide the clamp assembly away from the cooling core.

Loosen all four screws of the clamp assembly and remove the glass plate sandwich from it.

Push one of the spacers of the sandwich out to the side of the plates without removing it.

Gently twist the spacer so that the upper glass plate pulls away from the gel. Remove the plate. The gel will stick to one of the plates.

Float the gel off of the glass plate by inverting the gel and glass plate under fixative solution or blotting transfer buffer and agitate gently until the gel separates from the glass plate.

The gels proteins can now be transferred to membrane

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

Gloves as specified in the appropriate COSHH RA.

Exercise extreme caution for acid cleaning; wear safety glasses, a lab coat, and rubber gloves. Keep a container of $NaCO_3$ nearby to neutralize spills.

6.0 Spillages -

Always clean up any spills to both the Mini-PROTEAN II 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 Maintenance -

Mini-PROTEAN II cell chamber, inner core, clamps:	Rinse thoroughly with distilled water after every use.
Glass plates, spacers, combs:	Wash with a laboratory detergent, then rinse thoroughly with distilled water.
Glass plates (when more stringent cleaning is required):	Soak in a strong acid solution (chromic acid/sulfuric acid cleaning solution) for approx. 30 minutes and then rinse thoroughly with distilled water.

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/001, COSHH/013

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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