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Title: **Use and Maintenance of the BioRad Trans Blot Apparatus**

Version: **v2**

Author: **Peter Mullen**

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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 BioRad Trans Blot in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to routine use and maintenance of the BioRad Trans Blot within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the BioRad Trans Blot within the SASoM to comply with this SOP.

4.0 Procedure –

Principles of Operation:

The trans blot allows transfer of proteins, which have been separated on agarose gel, to be transferred to a membrane which in turn can then be used to probe for a variety of proteins by antibodies.

General Assembly of Unit for Transfer:

Always wear gloves when handling membranes to prevent contamination

Saturate two pieces of Thick Blot Paper and transfer membrane in 0.5x TBE buffer.

Equilibrate the transfer membrane for at least 10 minutes. The transfer membrane and blot paper must have the same dimensions as the gel for proper transfer to occur.

To assemble the transfer sandwich, hold up one piece of blot paper, allow the excess buffer to drain off, and lay it flat on the platinum anode. Using a clean pipette, roll out any air bubbles that may be trapped under the blot paper with a top-to-bottom and left-to right rolling motion.

Place the equilibrated transfer membrane on top of the blot paper and roll out the air bubbles.

Carefully place the agarose gel on top of the membrane, well side up. Make sure all the edges are aligned and air bubbles are rolled out.

Take the other piece of wetted blot paper, again allowing the excess buffer to drain off, and place it on top of the agarose gel. Roll out the blot paper to remove air bubbles and add approximately 15 ml of transfer buffer (0.5x TBE) on top to resaturate the sandwich. Be sure to remove any excess buffer that is present on the anode electrode surface.

Place the support frame around the gel/membrane/blot paper stack and connect the cathode electrode by locking it into place without disturbing the stack.

Place the safety cover onto the unit and plug the Trans-Blot SD cell into the power supply. Be sure to maintain normal polarity of the electrodes, i.e. red lead to red outlet and black lead to black outlet. Caution: Do not reverse the electrode polarity. This will damage the stainless steel cathode.

Turn on the power supply and run transfer.

Following transfer, turn off the power supply and disconnect the leads. Remove the safety cover and the cathode electrode. Discard the blot paper and recover the transfer membrane.

Rinse the membrane in 2x SSC. The transfer efficiency can be qualitatively monitored by staining the gel and checking for any remaining protein.

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 Trans Blot 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/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 –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name: Mary Wilson

Position: Laboratory Manager

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date: