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STANDARD OPERATING PROCEDURE

1. SOP for Gradiflow BF200	
2. Description: Electrophoretic fractionation of proteins	
3. Authorisation:	
<ul style="list-style-type: none"> • Authorisation provided by: • Custodian of Equipment: • Training required: • Supervision required for task: • Responsibility of Review of this SOP: 	Dr Valerie Wasinger Dr Valerie Wasinger Yes No Dr Valerie Wasinger
4. Resources:	
<ul style="list-style-type: none"> • Equipment: 	<ul style="list-style-type: none"> • 2L and 200mL beaker • 10mL disposable syringe cases • Cleaning cartridge • Separation cartridges • Stainless steel beaker (~5L) (provided with the instrument) • Mains power lead • Red and black connecting leads • GeneMate 250-4 Power supply pack
<ul style="list-style-type: none"> • Substances: 	<ul style="list-style-type: none"> • Separation buffer: 90mM Tris (Tris-(hydroxymethyl)-aminomethane) 10mM EACA (6-Aminohexanoic acid) • PBS • Other buffers can be used depending on your experiment. Refer to end of document for other buffer recipes.
<ul style="list-style-type: none"> • Other people: 	<ul style="list-style-type: none"> • N/A
<ul style="list-style-type: none"> • PPE: 	<ul style="list-style-type: none"> • Laboratory Coat • Safety Glasses • Gloves
5. Instructions:	
Environment	<ul style="list-style-type: none"> • Ensure bench area is dry and clean before starting • Check that there is enough space on the bench for the Gradiflow instrument AND the power supply. The instrument and the power supply pack will need to be close to a power outlet for connection.
Preparation	<p>Sample preparation</p> <ol style="list-style-type: none"> 1. Prepare your protein sample in a minimum of 10mL separation buffer. <p>Buffer preparation</p> <ol style="list-style-type: none"> 2. Prepare 2L of separation buffer

Step by Step
Instructions

Instrument Setup

1. Check that all tubing and electrical connections are properly fitted to the instrument
2. Make sure that the tubing for the stream pump cassette (located bottom right hand side of instrument) is connected to the outlet and inlet ports in the following configuration:
 - ⊙ Stream 1 (upstream) pump outlet (blue)
 - Stream 2 (downstream) pump outlet (yellow)
 - ⊙ Stream 1 (upstream) pump inlet (blue)
 - Stream 2 (downstream) pump inlet (yellow)
3. Lock the stream pump cassette over the spindle (this should click into place)
4. Plug in the Gradiflow BF200 to the power outlet

Setting the power pack (GeneMate 250-4 Power supply pack)

1. Plug in the electrodes of the power pack into the back of the separation unit
2. Set the following separation parameters: voltage- 250V; current- 500mA; time- 2.5 hours

Warning: When separating your sample, do not apply the voltage until all buffers are circulating throughout the instrument.

Note: The separation parameters are only a guide; they can be altered depending on your sample concentration.

Assembling the cartridge

Perform this if pre-packed cartridges for the Gradiflow BF200 are not available, otherwise, move on to the next section- **‘Inserting the cartridge’**

Caution: The pre-packed cartridges and membranes are stored in buffer that contains sodium azide (0.05%v/v)- wear gloves and safety glasses.

1. Before assembling, ensure all components of the cartridge to be used are clean. If not, 70% ethanol and Milli-Q-H₂O can be used to clean the cartridge parts
2. Place the housing clip onto a flat clean surface
3. Place the gasket into the housing clip
4. Place the restriction membrane on top of the gasket ensuring that the holes on either end of the membrane line up with those of the gasket (this may not be the perfect line-up but try to get it close as possible)

Step by step instructions

5. Place the grid onto the restriction membrane and make sure the **grooved channels** near the circular holes on either end of the grid (refer to picture in **DEFINITIONS** section) are **facing up**, i.e. the smooth side near the circular holes of the grid should be in contact with the restriction membrane. Make sure the holes at either end of the grid are aligned with that of the restriction membrane and gasket
6. Place the separation membrane on top of the grid
7. Place a second grid onto the separation membrane and make sure the **grooved channels** near the circular holes on either end of the grid (refer to picture in **DEFINITIONS** section) are **facing down** and therefore in contact with the separation membrane
8. Place the restriction membrane on top of the grid followed by the gasket. Ensure all holes on either end of the grid, restriction membrane and gasket are aligned
9. *OPTIONAL*: Place the retaining clip on top of the gasket to seal the cartridge

Inserting the cartridge

Warning: Ensure that ALL pumps and power are turned OFF before opening the separation unit and inserting the separation cartridge

1. Open the separation unit by rotating the knob fully anti-clockwise
2. Insert the cartridge into the unit label side up or if using own cartridge, the housing clip should be placed so that it sits at the bottom in the separation unit
3. Secure the cartridge in place by rotating the knob clockwise until it is **finger tight**.

Adding the sample and buffer

Caution: Make sure the stream and buffer pumps are TURNED OFF before loading or collecting any sample from the stream reservoirs.

1. Remove the ice container from the buffer reservoir and fill it with ice

Warning: Ensure the power supply is switched off when (re)filling the ice bucket with ice.

2. Fill the buffer reservoir with 1.8L of separation buffer (as prepared above) and place the ice container in the buffer reservoir

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Step by step instructions

3. For your stream reservoir, use 10mL syringe casings (or bigger if needed) and place them in the stream 1 (upstream) and stream 2 (downstream) ports located beneath their respective outlet tubing in front of the instrument. Ensure the outlet tubing of each stream is in their respective reservoir
 4. Before adding your sample, it is recommended that buffer be circulated through the instrument to check for any leakages. To do this, add the separation buffer (left over from the 2L prepared previously) to the stream 1 and stream 2 reservoirs and switch on the stream pump (green switch) followed by the buffer pump (red switch)
 5. Allow the buffer to circulate for approximately 5-10mins
 6. If no leaks occur, discard the buffer by stopping the stream pump and then redirect the outlet tubing from the stream reservoir to another tube
 7. Switch on the stream pump and allow the buffer to empty from the stream reservoir. Switch off the stream pump before loading your sample
- Note: If leaking occurs, tighten the separation unit a little further or change the cartridge, which may be damaged. Leaks are usually observed from the separation unit and are also indicated by a drop in buffer levels within the stream reservoir.*
8. Load your sample into either stream 1 or stream 2 reservoir
 9. Load the same volume of buffer into the other stream reservoir
- Protein separation**
1. Switch on the stream pump and the buffer pump
 2. Ensure all buffers are circulating
 3. Press **START** on the power supply pack
- Note: The system must remain cool during separation so there may be a need to refill the ice bucket. REMEMBER to turn OFF the voltage before refilling the ice bucket.*
- Collecting the sample during separation**
- To monitor the separation, sample aliquots can be collected during separation.
1. Stop the voltage
 2. Using a pipette, collect the required amount from stream 1 and stream 2 reservoir
 3. Restart the voltage

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Step by step instructions	<p>Collecting the final fractions</p> <p>Once the separation is complete the voltage is switched off automatically. To collect the final fractions:</p> <ol style="list-style-type: none">1. Switch off the stream pump2. Redirect the outlet tubing of stream 1 and stream2 from their stream reservoirs into a suitable collection tube e.g. 10mL conical falcon tubes3. Switch on the stream pump and allow for stream 1 and stream 2 to EMPTY completely from the stream reservoirs4. Store stream 1 and stream 2 fractions appropriately <p>Post-separation washes (RECOMMENDED)</p> <p>To collect the residual protein that may still be present in the instrument (e.g. proteins stuck to the membrane and residual protein present within the stream tubing) after separation, PBS washes are recommended.</p> <ol style="list-style-type: none">1. Add 10mL PBS to stream 1 and stream 2 reservoirs2. Switch on the stream pump and allow the PBS to circulate for 10 minutes3. Switch off the stream pump and collect the PBS wash4. Repeat for a total of 3 x 10 minutes washes5. OPTIONAL: The PBS washes can be added to the final fractions collected in the previous section <p><i>NOTE: No voltage is applied during the washing step and so the power supply pack can be removed from the instrument.</i></p>
Clean up and Waste Disposal	<p>Cleaning the instrument</p> <ol style="list-style-type: none">1. Remove the ice bucket from the buffer reservoir and empty2. Pull the buffer outlet tubing from its holder located above the buffer reservoir on the right hand side of the instrument and direct the tubing to a waster container (the ice bucket be used for this)3. Switch on the buffer pump and allow the buffer reservoir to empty4. Switch off the buffer pump5. Empty the stream reservoirs if not already done so6. Discard the buffer into the non-halogenated waste container <p>Caution: Make sure ALL pumps are switched OFF before opening the separation unit.</p> <ol style="list-style-type: none">7. Loosen the separation unit and remove the cartridge8. Dispose the cartridge into the biological waste bin

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Clean up and Waste Disposal	<p>9. To clean the buffer reservoir and stream tubing, insert the CLEANING CARTRIDGE into the separation unit and tighten.</p> <p>Cleaning the buffer reservoir</p> <ol style="list-style-type: none"> 1. Direct the buffer outlet tubing to a waste container 2. Fill the buffer reservoir with 2L of Milli-Q water 3. Switch on the buffer pump and allow the buffer reservoir to empty 4. Repeat steps 2 and 3 for a total of 3 x 2L rinses <p>Cleaning the streams</p> <ol style="list-style-type: none"> 1. Place 20mL syringe casings into the stream 1 and stream 2 ports and fill with Milli-Q water 2. Switch on the stream pump and allow the water to circulate for 1 minute 3. Discard the rinse solution- REMEMBER to switch off the stream pumps when filling or emptying the stream reservoirs 4. Refill the reservoirs with water and repeat for a total of 3 x 1 minute rinses <p>Depending on the sample run, the instrument should be cleaned thoroughly at least once a week. To do this:</p> <ol style="list-style-type: none"> 1. Fill stream 1 and stream 2 with Milli-Q water and allow it to circulate for 1 minute 2. Discard water 3. Fill stream 1 and stream 2 with 20% ethanol and allow it to circulate for 1 minute. Discard to non-halogenated waste container 4. Rinse the streams with Milli-Q water for a total of 3 x 1 minute rinses 5. Remove cleaning cartridge and store for later use
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6. Key hazards Refer to <i>Risk Assessment for Gradiflow BF200</i>	Risks from Hazard	Control Measure including PPE
<ul style="list-style-type: none"> • Chemical (buffer) Spills 	<ul style="list-style-type: none"> • Chemical exposure to skin, eyes, respiratory system • Chemical burns 	<ul style="list-style-type: none"> • Laboratory coats, gloves, safety glasses, face mask • First Aid Kit • Eye wash stations
<ul style="list-style-type: none"> • Electrical hazards 	<ul style="list-style-type: none"> • Shock • Fire 	<ul style="list-style-type: none"> • Equipment testing and tagging • First Aid Kit • Fire Extinguisher

7. Emergency Procedures and Shutdown

- Inform supervisor and first aid officer/fire warden
- For large scale emergencies dial x56666
- An eye wash station is located above the sink besides the Gradiflow BF200 (M312). There are other eye wash stations in room M304

- First Aid Kit in room M310 and M304
- Safety shower in room M307
- Hose reel and CO₂ fire extinguisher just outside M305
- Fire extinguishers available in the BMSF: CO₂ in rooms M305 and M307; Dry Chemical extinguisher is located beside the electrical cupboard and store room

8. Legislative References *List all legislation, codes of practice and Australian standards affecting this SOP*

9. Definitions

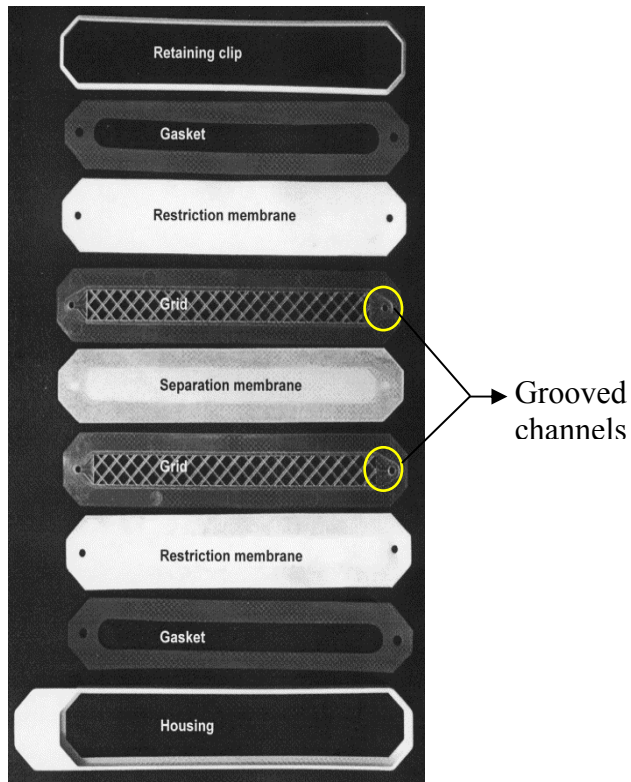
Assembling the cartridge

(Diagram: Rylatt, D.B., Napoli, M., Ogle, D., Gilbert, A., Lim, S., Nair, C.H., 1999, 'Electrophoretic transfer of proteins across polyacrylamide membranes', *Journal of Chromatography A*, 865: 145-153.)

Step 9



Step 1



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Gradiflow BF200

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