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STANDARD OPERATING PROCEDURE

1. SOP for Gradiflow MF10 (prototype)	
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: 	<ul style="list-style-type: none"> Dr Valerie Wasinger Dr Valerie Wasinger Yes No Dr Valerie Wasinger
4. Resources:	
<ul style="list-style-type: none"> • Equipment: • Substances: • Other people: • PPE: 	<ul style="list-style-type: none"> • 500mL beaker • Separation cartridges- 6 chamber • 5kDa restriction membranes • Separation membranes of different pore sizes- e.g. 500, 150, 125, 65 and 45kDa membranes • Mains power lead • Red and black connecting leads • GeneMate 250-4 Power supply pack • 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. • N/A • 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 separation buffer to a total volume of 100-400µL <p>Buffer preparation</p> <ol style="list-style-type: none"> 2. Prepare 500mL of separation buffer

Step by Step
Instructions

Instrument Setup

1. Plug the Gradiflow MF10 to a power outlet
2. Connect the electrodes of the power supply pack to the instrument (located on the left side)
3. Set the following separation parameters: voltage- 250V; current- 500mA; time- 90 minutes

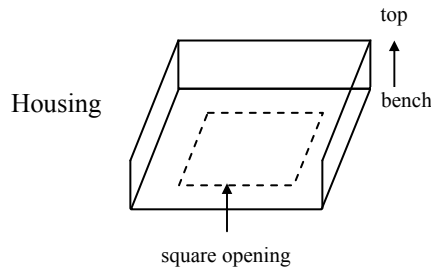
Warning: *When separating your sample, do not apply the voltage until all buffers are circulating throughout the instrument.*

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

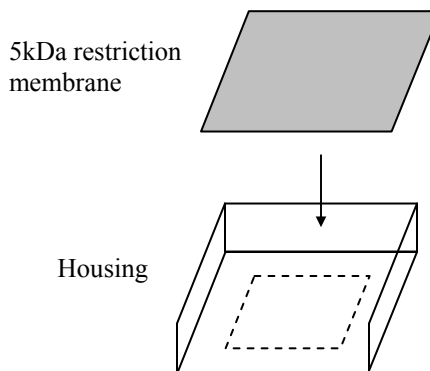
Assembling the cartridge

Caution: *The 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 used are clean. If not, 70% ethanol and Milli-Q-H₂O can be used to clean the cartridge parts
2. Place the housing of the cartridge onto a flat clean surface. The side arms of the housing should be facing up and the square opening facing the bottom

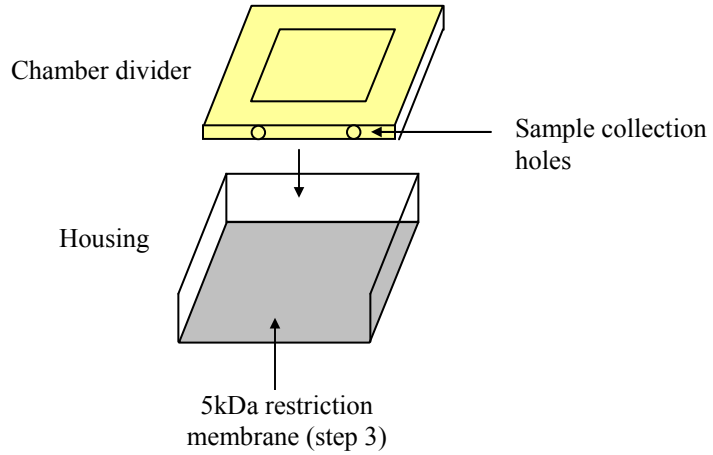


3. Cut the 5kDa restriction membrane and lay it into the housing ensuring that it covers the square opening completely. Make sure the membrane fits along the side walls of the housing

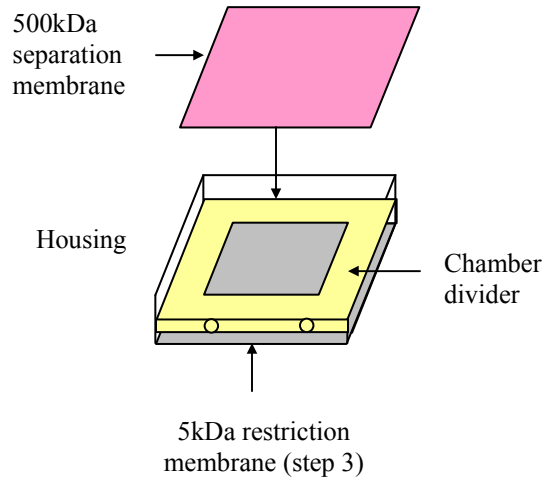


Step by step instructions

4. Place a chamber divider on top of the 5kDa restriction membrane ensuring the sample collection holes are facing the outside of the cartridge



5. On top of the chamber divider place a separation membrane e.g. 500kDa ensuring it covers the chamber divider's square opening completely



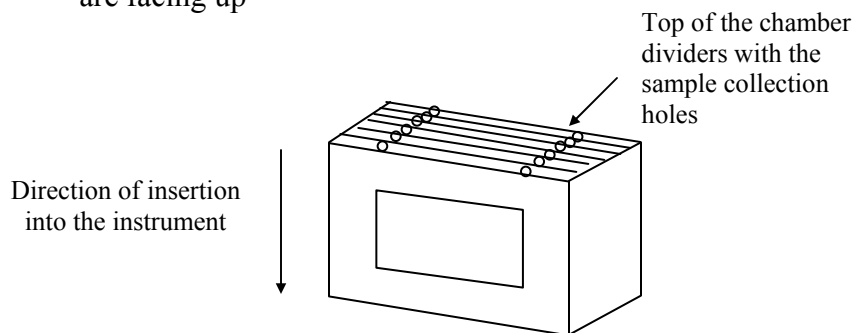
6. Place a chamber divider on the separation membrane followed by the next separation membrane e.g. 125kDa. Repeat this with the remaining separation membranes with a chamber divider placed between each membrane. Make sure membrane covers completely the square opening of each chamber divider
7. Place the 5kDa restriction membrane on top of the last (6th) chamber divider
8. Secure the cartridge stack by placing the backing of the cartridge on top of the 5kDa restriction membrane. BE CAREFUL when handling the assembled cartridge- it can easily fall apart.

Step by step instructions

Inserting the cartridge

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

1. Open the separation unit by rotating the knob fully anti-clockwise
2. In the membrane cartridge upright so that the sample collection holes are facing up



3. Secure the cartridge in place by rotating the knob clockwise until it is **finger tight**

Note: Make sure the chambers and membranes do not slide up out of the housing once secured into the separation unit. If this occurs, re-tighten the separation unit but do not do this too often otherwise you risk damage to the membranes. If you cannot secure the cartridge it may be that you will need to reassemble the cartridge with new membranes.

Adding the sample and buffer

1. Place the buffer inlet and outlet tubing located at the front of the instrument into a beaker containing 300-500mL of separation buffer. There should be enough buffer for constant circulation throughout the system. If not, simply add more buffer
2. Using a gel loading pipette tip, load your sample (100-400 μ L) via the sample collection holes into the chamber closest to the cathode (-) (towards front of instrument) or the anode (+) (towards the back of instrument)
3. Fill the remaining chambers with the same volume of buffer

Protein separation

Caution: Do not start the protein separation or the buffer pump until the cartridge is inserted and the buffer reservoir is set up.

1. Fill the back of the instrument with ice

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Step by step instructions

2. Turn on buffer pump by flicking the switch **UP**
3. Ensure the buffer are circulating (the buffer should be flowing continuously out of the outlet tubing)
4. Press **START** on the power supply pack

Note: The system must remain cool during separation so refill the ice reservoir when needed

Collecting the sample after separation

Once the separation is complete the voltage is switched off automatically. To collect the final fractions:

1. Switch off the buffer pump
2. Using a gel loading pipette tip collect the sample from each of the chambers and transfer to an eppendorf tube

Post-separation washes (RECOMMENDED)

To collect the residual protein that may still be present in the chambers after separation, PBS washes are recommended.

1. Add PBS at an equal volume to the sample added earlier to each chamber
2. Switch on the buffer pump and allow the PBS to wash for 5 minutes
3. Switch off the buffer pump and collect the PBS wash
4. Repeat for a total of 3 x 5 minutes washes
5. **OPTIONAL:** The PBS washes can be added to the final fractions collected in the previous section

NOTE: No voltage is applied during the washing step and so the power supply pack can be removed from the instrument.

Clean up and Waste Disposal

Cleaning the instrument

1. Leave the cartridge in the instrument
2. To empty the buffer from the instrument, flick the buffer switch completely **DOWN** and hold until all buffer has emptied. Discard
3. Place the inlet and outlet tubing into a beaker of Milli-Q water and allow to circulate throughout the instrument for 1 minute. Discard water
4. Repeat step 3 for a total of 3 x 1 minute rinses

Caution: Make sure ALL pumps are switched off before opening the separation unit.

5. Loosen the separation unit and remove the cartridge
6. Wipe the inside of the separation unit with 70% ethanol

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Clean up and Waste Disposal	<p>Cleaning the cartridge</p> <ol style="list-style-type: none"> 1. Take apart the cartridge and dispose the membranes into a biological waste container 2. Rinse the cartridge pieces thoroughly with Milli-Q water 3. Wipe down the cartridge pieces thoroughly with 70% ethanol 4. Allow to dry and store appropriately 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