Dialysis

Sample Preparation

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Dialysis Kit

1. Pierce 96-well Microdialysis Plate (Thermo)[1]
   - Sample volume 10-100 µL
   - 5K, 10K MWCO available
   - Rapid dialysis in 2-4 hours
   - Suggested for testing different buffer conditions or multiple samples

2. Slide-A-Lyzer MINI Dialysis Unit (Thermo)[2,3]
   - Best for 10-100 µL (physical capacity is 500 µL
   - 2K, 3.5K, 7K, 10K, 20K MWCO available

Using the Pierce 96-well Microdialysis Plate (Thermo)

See Procedure Summary from Thermo User Guide (Appendix A)[1]

Recommendations from the Wysocki Lab:

- To prevent membrane contamination, handle the device at the top or sides using gloves.
- To rinse the dialysis device, add 100µL of dialysis buffer to each microdialysis device by slowly adding the buffer through the round opening of the device. Remove the buffer from the device by setting the pipette volume to 140µL, inserting the pipette tip into the round opening and slowly aspirating the buffer. Do not let the membrane become dry. Check the volume of removal, if it is much larger than 100 µL, the membrane might be broken.
- After loading the sample, confirm that the sample is settled at the bottom of the device, especially when loading a small volume (e.g., 10µL), by carefully pushing the sample down with air through the pipette.
- Cover the top (sample loading portion) of the device with laboratory film.
- A typical dialysis using two buffer changes takes less than 5 hours to remove salts (e.g., 1M NaCl); however, dialysis time will vary depending on the salt and small molecule concentrations. A typical dialysis procedure is as follows: dialyze for 2 hours at room temperature or 4°C; change the dialysis buffer and dialyze for another 2 hours; if needed, change the dialysis buffer and dialyze overnight. To change the buffer, move the microdialysis device into a new deep-well plate channel or use a new microcentrifuge tube.
- For sample density≥1.150g/mL(e.g., 4.1M (NH4)2SO4, 45% sucrose or 8M GdnHCl), use less than 50µL sample volume. Performing serial dialysis using buffers with decreasing concentrations of solutes (salt) will prevent the osmotic pressure from overfilling the device (e.g., dialyze a 5M NaCl sample against a buffer with 0.5M NaCl). 5-8 times of changing buffer is suggested.

Using the Slide-A-Lyzer MINI Dialysis Unit (Thermo)

See Procedure Summary from Thermo User Guide (Appendix B)[2]

Recommendation from Wysocki Lab:

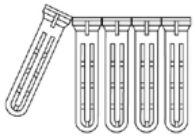
- During soak step, check whether the membrane is broken or not before injection of the sample.

References

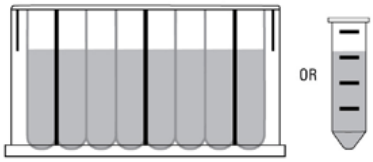
1. Pierce® 96-well Microdialysis Plate instructions
2. Slide-A-Lyzer™ MINI Dialysis Unit instructions
3. https://www.thermofisher.com/order/catalog/product/69550

Appendix A

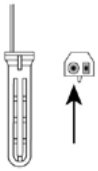
Procedure from Thermo User Guide for using the Pierce 96-well Microdialysis Plate (Thermo)[1]



1. Remove one or more devices, as needed. If only one device is required, break it carefully from the 8-segmented cartridge.



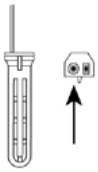
2. Add dialysis buffer to a deep-well plate (≤ 1800µL) or a 2mL microcentrifuge tube (≤ 1400µL) and set aside.



3. To load each device, insert an upright pipette tip filled with sample into the round opening (see arrow). Slowly add the sample (10-100µL).

4. Place device into the deep-well plate or 2mL microcentrifuge tube containing buffer.

5. Dialyze to remove low molecular weight compounds (1 hour to overnight). Change dialysis buffer as required. Shake plate gently on a plate shaker (optional).



6. Remove device from plate or tube and recover sample. Set pipette volume to 140µL, insert upright pipette tip into round opening of device and slowly withdraw the sample.

Appendix B

Procedure from Thermo User Guide for using the Slide-A-Lyzer MINI Dialysis Unit (Thermo)[2,3]

1. To prevent contamination, do not touch the membrane with ungloved hands.
2. Place the unit into the float so that the bottom of the dialysis unit is in contact with the dialysate. Always make sure that the volume level of the sample is at or above the level of the dialysate. If the volume level of the sample is lower than the level of dialysate, hydrostatic pressure will force dialysate into the unit, diluting the sample.
3. Soak the Slide-A-Lyzer MINI Dialysis Unit in 1L of water for 15 minutes.
4. Although the physical capacity of the unit is 500µL, for best results, apply a sample volume of 10-100µL.
5. Cap the Slide-A-Lyzer MINI Dialysis Unit and place in a flotation device.
6. Use a low speed setting on a stir plate so that the flotation device is not submerged.
7. Typical dialysis time to obtain equilibrium is 10 minutes to 2 hours using a dialysate volume of 0.5-1L.
8. For the best volume recovery, collect the sample from the corner of the Slide-A-Lyzer MINI Dialysis Unit.