Diafiltration

Sample Preparation

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12 january 2019

Buffer exchange into a mass spectrometry compatible electrolyte such as ammonium acetate can be accomplished by diafiltration (also known as ultrafiltration). Diafiltration is a convenient method for concentrating a protein sample after dialysis. The molecular weight cut-off (MWCO) chosen for diafiltration should be at most half the estimated molecular weight of the smallest protein intended for analysis. For example, if you are trying to use diafiltration on a 60 kDa protein, choose a 30 kDa MWCO diafiltration device. It is important to keep in mind that a MWCO does not truly separate by molecular weight, but by hydrodynamic radii, as this may impact the recovery of proteins with small hydrodynamic radii. Another consideration is the filter material. Some proteins will stick to cellulose, there is also PES (polyethersulfone) which may be a more suitable choice. When working with RNA or DNA a good rule of thumb is to choose a membrane with a MWCO that is one sixth the estimated molecular weight of the target analyte.

Buffer Exchange Protocol (0.5 mL device)

1. Deposit 500 uL of water and centrifuge for 15 minutes. Remove excess water by pipetting it out, or by inverting the diafiltration device and centrifuging for one minute at 1000 x g. This removes excess glycerine.
2. Load sample into suitable diafiltration device, add enough ammonium acetate to bring solution volume to 0.5 mL
3. Spin at the specified speed (for amicon 0.5 mL ultrafiltration 14,000 x g) for 15-30 minutes.
4. Repeat 2-10 times. Three repeats is sufficient for most applications, although this can be different sample to sample.

Commonly Used Products

- Amicon Ultra-0.5 mL
- Vivaspin®

Sample concentration

1. Simply load the sample into the device and spin at 14000 xg for 45-60 minutes –or until the desired volume and concentration are achieved.
2. To recover the sample place the device into a new collection tube upside down and centrifuge for one minute at 1000xg.
    - Alternatively, a gel loading pipette tip can be used to collect the sample and place into a microcentrifuge tube. The collection tubes sent with the diafiltration device are not perfectly conical at the bottom which can lead to downstream sample loss.

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