DIALYSIS OF FBS TO REMOVE AMINO ACIDS

Reagents	Materials
1xPBS (8-liters) sterile ddH_2O FBS	Dialysis Tubing 3500 MWCO (Fisher Scientific #21-152-9 is 5cm wide) 4-liter beaker containing 4-liters of ddH_2O (with a stir bar) stirbar & stirplate Dialysis clips (7.5cm) Funnel (sterile)

General information on dialysis:

• Handle tubing only with powder-free gloves to avoid getting skin oils on membrane.

• Dry/packaged dialysis tubings generally contain some heavy metals and glycerin. Repeated soaking in water removes the glycerin; boiling the tubing will also remove the glycerin.

• Recommended buffer volume is 10-times the volume of the sample in the tubing.

• Although diffusion rate is somewhat reduced in the cold, almost all protein dialysis is conducted at 4ºC to enhance protein stability.

⊡ If not used immediately, tubing should be stored covered at 4ºC in water containing 0.1% sodium azide. Once wetted, the membranes should not be allowed to dry out.

Procedure

1) Calculate the volume of amino-acid free FBS needed. Fisher tubing #21-152-9 estimates 6.7ml/cm tubing. Add an extra 5-10cm to allow for the dialysis clips.
2) Place tubing in 4-liter beaker containing 4-liters of ddH_2O with a stir bar. Make a foil lid and autoclave tubing in beaker.
3) Wearing gloves, Remove tubing with plastic forceps (careful not to puncture membrane!).
4) Clip the bottom of the dialysis tubing with a weighed dialysis clip. Take care to make sure there are no folds in the membrane.
5) Fill tubing about 3/4 full with sterile ddH_2O. Gently squeeze tubing from the top to inspect for leaks in the tubing or at the bottom of the clip.
6) Pour out ddH_2O and use a funnel to add FBS. Clip top of tubing with non-weighted dialysis clip. Check for leakage at both ends by applying a small amount of pressure to the sample area.
7) Pour out ddH_2O from the beaker and add 4-liters sterile 1XPBS.
8) Place dialysis tube in the beaker and replace foil lid. Make sure that the bag is completely immersed.
9) Stir tubing gently on a stir plate in the coldroom for ~4-6 hours.
10) Pour off old 1xPBS buffer and add remaining 4-liters of 1XPBS and gently stir overnight.
11) Remove the dialysis bag from the buffer and rinse the exterior with distilled water. *If the bag is extremely taut as a result of buffer infiltration, do not remove either dialysis clip; instead put one end of the tubing into a beaker large enough to hold the entire sample, then puncture the tubing near the lower knot with a needle and allow the sample to drain directly into the beaker.*
12) Filter sterilize dialyzed FBS using a Millipore 22μm filter unit.

Ref: Current Protocols in Protein Science, *Desalting, Concentration, and Buffer Exchange by Dialysis and Ultrafiltration,* **Unit 4.4, 2**001 John Wiley & Sons, Inc.