ADAMTS-1 purification

1. Growth cells expressing ADAMTS-1 in 150 mm plate with DMEM 10% FBS or FCS.

2. Split enough times so that you can seed cells into 24cmX24cm Nunc/Nalgene TC square plates at 50% confluency. (I usually do this for 4-5 plates to get enough ADAMTS-1 each time.)

3. When cells get to about 75-85% confluency, remove DMEM containing serum, and wash with serum free DMEM 3 times to remove as much serum as possible. (Serum contains alpha-macroglobulin which bind to and inactivate ADAMTS-1. Incubate the last rinse for 30min to 1 hour before removing.)

4. After final rinse, replenish with serum free DMEM containing 5 ug/ml of HEPARIN. Use 70 ml total volume/plate. It is important to make sure that the plates sit level in the incubator as with such small media volume, slight tilting of the plate will result in cells that will be without media. (note: HEPARIN is needed to prevent processing of ADAMTS-1 to the 65 kDa form. If you wish to collect 65 kDa ADAMTS-1, do not add HEPARIN.)

5. After 36 hours collect media and filter with 0.4 um pore size PES membrane to remove cells. This is very important as cell debris will clog the BIO-RAD biologic chromatography machine.

   (once collected the media can be frozen until ready to purify.)

6. Remove any column that's hooked up to the BIO-RAD machine and flush Biologic with water. This will remove any salt precipitates.

7. Equilibrate machine with 150 mM NaCl /10 mM HEPES pH 7.4 and attach Heparin column.

8. Equilibrate Heparin column with 20 ml 150 mM NaCl/ 10 mM HEPES pH 7.4 and feed ADAMTS-1 containing media through column at 0.5 – 1 ml/min flowrate.

9. Then wash with 550 mM NaCl/ 10 mM HEPES pH7.4, 30 ml at 1 ml/min Flowrate

10. Elute with 1 M NaCl/ 10 mM HEPES pH 7.4 at 1 ml/min, collecting 3 ml fractions.

11. Looking at the UV peaks pool the fracations that contains the proteins together to dialysis out the high salt. Usually, fraction 13 and 14 is where 87kDa comes out, and fraction 3 and 4 is where 65 kDa comes out.

12. Dialyze fractions with 10,000 MW Pierce dialysis cassettes in 250 mM NaCl, 10 mM HEPES pH 7.4, 1 mM MgCl2 and 1 mM CaCl2. aliquot proteins and store at –80ºC.