SEPARATING MEMBRANE-TARGETED MOLECULES USING GEL FILTRATION

CL 2B Sepharose has an exclusion limit of 40,000 kDa. This means that anything larger than this will come out of the column in the void volume (this includes membranes and anything that is integrated/secreted in them). Smaller molecules will be retained in the gels beads and more out of the column more slowly thus will appear in the later fractions.

Therefore,

A) excluded fractions contain those molecules which are too large to go through the pores and move between and around the beads (> 40,000 kda) (= mbs + associated molecules). This will be the void volume.

B) retained fractions contain those molecules which are small enough to pass through the gel pores (< 40,000 kda) (= non mb associated molecules).

Void volume is ~ 40% of bed volume.

Use a 1cc syringe to set up a column of :

- bed volume = 700lambda
- void volume = 280lambda

The drops you collect will depend on drop volume.

ie: if fraction size = 50(Therefore, void comes in fractions 6, 7. collect 5, 6, 7 to be safe.

For initial calibration of columns the appropriate controls (without membranes) should be done and up to 16 drops collected separately and run out (2-5() on SDS-PAGE.

For +mbs samples there should be a peak of protein at the void fraction.

For -mbs samples most of the protein should be in the later fractions.