

DE-52 COLUMN CHROMATOGRAPHY.

Use DE-52 directly from the bottle. Make a slurry by suspending in starting buffer 0.01 M Tris/0.01 M NaCl pH 8.1 (pH 7.6 for FITC purification) in a beaker. *see recipe* *8.1 in 10x stock*

pH of slurry will be too high so adjust with 1N HCL and pH meter, stir well. Fill column approximately 1/4 full with starting buffer allowing some to run out at the bottom to clear all the air from the system. Then clamp off with gate clip. Pour slurry into column and allow to partly settle --then allow column to drain very slowly to speed packing. When packed, wash well with starting buffer approximately 20 x bed volume. Compare pH and conductivity of column effluent with that of starting buffer. Drain column to top of bed.

Fill gradient-maker high salt first, fill connector tube by releasing clip momentarily, clear all trapped air (very important) allowing a small amount of high salt/buffer to enter low salt chamber. Fill low salt and fill delivery tube to column.

Load sample allow to enter bed. Add small vol. of starting buffer allow to enter bed. Add further a small vol. of starting buffer. Stopper column, remove all dips, start fraction collector and magnetic stirrer. Collect 2-3 ml vol. fractions so as to be able to use autocuvette on spectrophotometer. Read samples at OD₂₈₀. *flow rate 20 ml/min low FITC*

Extra Hints:

1 ml serum/gm dry wt. (from bottle (75% water really)).

10 mg γ glob/ml serum

Derek's rule of thumb: max 20 OD₂₈₀ units/ml wet bed. For DE-52, 1 1/2 ml wet bed \sim 1 g from bottle (75% H₂O)

Total volume of gradient: use 8 or 10x vol. of bed.

Example: If have 40 mg prot. in sample, equiv. to 4 ml serum, or 4 gm DE-52. Column size: 1/2" diam. 8-10" long.

Also gradient size imp. Don't use too much, or will dilute sample. for 40 mg, use ~~300~~ 100 ml total volume (both buffer chambers)

Flow rate of buffer must be slow better than 1 drop every 6-12 secs. depending on drop size, column size, gradient size.

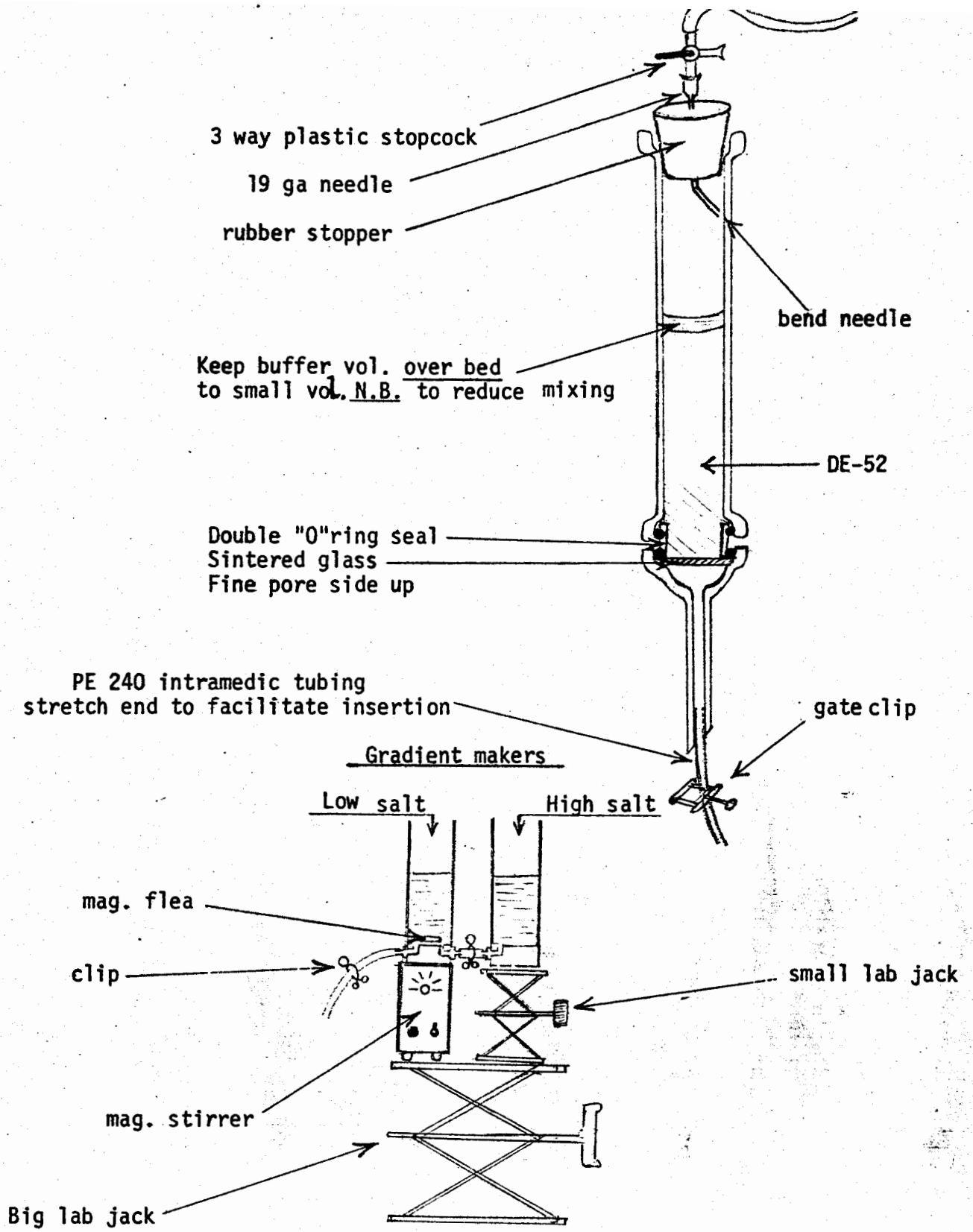
Buffer: for separating Ig

→ 0.01M Tris/0.01M NaCl 0.01M Tris/0.3 M NaCl pH 8.1 (Add 16.95 gms NaCl/litre of 1x low salt buffer.)

For FITC or RITC

→ 0.01 M Tris/0.01 M NaCl 0.01 M Tris/0.5 M NaCl pH 7.6 (Add 28.64 gm NaCl/litre of 1x low salt buffer.)
low salt *High Salt*

20 OD₂₈₀ units



Flow of column may be varied by raising or lowering Big Jack.