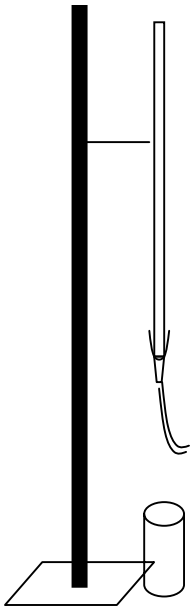
Sephadex G50/G100 Gravity Columns



I. Procedure:

A. Assemble column as follows:

1. Break off the mouth end of disposable pipette.
2. Place nitex circle over the end of pipette
3. Push yellow tip over nitex.
4. Secure yellow tip with parafilm.
5. Place intramedic tubing onto yellow tip.
6. Secure with parafilm.
7. Secure column onto a support; i.e., a stand and clamp.
8. Place a beaker under column to catch column eluate.
9. Pinch tubing closed with hemostats.
10. Add several mls of running buffer.
11. Add the Sephadex to the column.
12. Allow the Sephadex to settle into the column by gravity.
13. Open the column and allow the buffer to flow through the Sephadex.

Do not let the buffer drain below the top of the Sephadex; i.e., don't let the column dry out otherwise you will have to start over.

14. Continue to add more Sephadex until you have ~5ml of settled bed volume.
15. Wash column with ~3 volumes of running buffer.
16. Drain column so that there is about 0.5ml of buffer covering the bed.
17. Pinch/clamp the column.

At this point the column is ready to use or you may store it (a day or so) and use it later.

B. Loading the sample. (Sample volume is usually 50µl of reaction plus 50µl of Stop buffer)

1. Drain the buffer above the column such that no buffer is evident at the top of the Sephadex. (Again don't let the Sephadex dry out.)
2. Apply sample to the column such that it runs down the side of the pipette as one complete stream.
3. Allow sample to enter column.
4. Gently add 1ml of running buffer.
5. Fill the remainder of the column void with running buffer.
6. Follow the sample down with a Geiger counter.

The incorporated label runs with the blue dextran dye (fast) and the unincorporated label runs with the Orange G (slow). It will take 7-10 minutes for a radiolabeled fragment to reach the bottom of the column when using G50. G100 will take 10-15min.

7. Collect just the "hottest" fraction, generally ~0.5-1.0ml into a microfuge tube.
8. You can determine the specific activity of the probe by counting 1µl of the collected fraction.

Materials

1. Sephadex G50

a) Aliquot a small quantity of Sephadex G50 or G100 to a clean glass bottle.
b) Cover with ddH_2O
c) Autoclave

2. Running buffer:

	200mls	Final Conc
a) 1M Tris	2.0ml	10mM
b) 0.5M EDTA	0.4ml	1mM
c) 5.0M NaCl	0.2ml	5mM
d) 10% SDS	2.0ml	0.1%
e) dH_2O	196ml	

3. 2X Nick Stop Dye Buffer:

	10ml	Final Conc
a) 1M Tris	0.2ml	
b) 0.5M EDTA	40µl	
c) 5.0M NaCl	20µl	
d) 10% SDS	0.2ml	
e) Blue Dextran	0.1g	1.0%
f) Orange G	0.003g	0.3%
g) dH_2O		

(Note: this is not the same Nick Stop Dye I got from Diers/Osborne; I adjusted concentrations so that they would be consistent with the running buffer; i.e., Diers's recipe is 50mM Tris, 100mM EDTA, 1% SDS and no NaCl)

4. Disposable 5 or 10 ml pipette.

5. Piece of nitex cut to the size of a dime.

6. Short piece of 0.047 X 0.067 intramedic tubing (VWR cat# 63019-149)

7. Hemostats

8. Sterile yellow and blue tips and microfuge tubes

9. Rack for tubes