

PREPARATION OF SEPHADEX G-25 AND SEPHACRYL S-300

Adapted from BV's Cookbook, TCH 1/27/02

Preparation of Sephacryl S-300:

1. Add ~ 700 ml water to 300 ml Sephacryl S-300 HR (Pharmacia 17-0599-10) (300 ml = the entire contents of two bottles of 150 ml each).
Swirl and sit overnight at 4°.
2. Pour out sup, add ~300 ml TE (10 mM Tris, 1 mM EDTA, pH 7.5).
Swirl and sit overnight at 4°.
3. Volume of bed should be ~300 ml.
Pour out sup, add 200 ml TE (total volume is ~500 ml).

Preparation of Sephadex G-25

1. Add 100 g dry Sephadex G-25 (DNA grade, Pharmacia 17-0572-01) to 1000 ml of water.
Swirl and sit overnight at 4°.
2. Pour out sup, add ~2000 ml water.
Swirl and sit overnight at 4°.
3. Pour out sup, add ~ 700 ml TE (10 mM Tris, 1 mM EDTA, pH 7.5).
Swirl and sit overnight at 4°.
3. Volume of bed should be ~450 ml.
Pour out sup, add 250 ml TE (total volume is ~700 ml).

Note:

- a. To prepare with SDS, add 1 ml 10% SDS to 100 ml of the final resin preps.
- b. To check, centrifuge 5 minutes as usual in QS-GS column; height of bed volume should be 1.6 -2 cm.