IMMOBILIZATION ON CYANOGEN BROMIDE ACTIVATED SEPHAROSE

- Must have protein free of buffers that contain amino groups (that includes Tris) as these groups will attach to the resin and interfere with the coupling reaction.
- 1g dry gel gives about 3.5ml swollen gel which can bind 30-200nmol ligand
- want to aim for 80% binding capacity i.e. 160nmol. Sometimes extensive coupling will lead to loss of antibody binding sites. In which case, stop coupling reaction at 70% efficiency.

1. Gently suspend 1g CNBr-activated Sepharose in 1mM HCl (86µl concentrated in 1L) gently on a sintered glass funnel. Stir slurry with glass rod for about 20 minutes until matrix is swollen, then apply vacuum to remove liquid.

2. Wash matrix with 3 x 100ml 1mM HCl. Wash to dryness each time.

3. Wash matrix with 50ml **coupling buffer**.

4. Dilute 30-200 nmol ligand in 15ml **coupling buffer**. Remove 100µl aliquot for later analysis.

5. Quickly transfer activated Sepharose to a flask containing the ligand solution.

6. Shake gently overnight at 4°C.

7. Filter matrix on sintered glass funnel and collect flow through. Remove 10µl for gel analysis (compare to 10µl of starting solution to estimate binding efficiency). Keep remainder until you know whether the coupling reaction has worked.

8. Wash matrix with 100ml **coupling buffer** on sintered glass filter.

9. Transfer matrix back to flask and incubate with 50 ml **blocking buffer** at room temperature for two hours or 4°C overnight.

10. On sintered glass filter, wash matrix with 50 ml **coupling buffer** and then 50 ml **acetate buffer**.

11. Wash with 4 cycles of 50ml **coupling buffer** followed by 50ml **acetate buffer**.

12. Finish the washes with coupling buffer containing 0.02% azide. The matrix is now ready for storage.

Coupling Buffer	**100ml**
0.1M $NaHCO_3$	10ml 1M
0.5M NaCl	10ml 5M
pH8.3	

Blocking Buffer	**100ml**
1M ethanolamine	
pH8.0	

Acetate Buffer	**100ml**
0.1M sodium acetate	3.3ml 3M
0.5M NaCl	10ml 5M
pH4.0	