CONJUGATION OF DYNABEADS (Dynabeads M270 Epoxy)

1. Resuspend entire vial of Dynabeads in 16ml of 0.1M NaPhosphate buffer, pH 7.4 - 2 x 10^{10} beads

2. Vortex bottle 30 seconds

3. Put 4ml of beads suspension into four 15 ml Falcon tubes (35 / 2097)

4. Wash the rest of the beads in the glass vial with 1-2ml of 0.1M NaPO4 buffer. Combine with the rest.

5. Shake slowly for 10 minutes on a shaking platform (FOR EXAMPLE USE THE NUTATOR)

6. Meanwhile prepare the antibody (ICN 55944). Dilute the lyophilized powder (50mg) in 3ml of DDH20 (resulting concentration 17mg/ml).

7. Mix buffers in the following order (this will be **AB mix**)
    1) **Add 1175 ul - Rabbit IgG (17mg/ml) IMPORTANT: THE RABBIT IgG should spin for 10 minutes at 14K. Save the supernatant and discard the pellet.**
    2) 12.2ml –0.1M Sodium Phospate pH7.4
    3) 6.65ml- 3M Ammonium Sulfate-Add slowly shaking the tube a bit.

8. Filter the resulting solution.

9. Transfer the 4 ml beads suspensions from the 4x 15 ml screw-cap Falcons to 4x 5 ml snap-cap Falcons ((35 2063). These tubes fit nicely in the Magnet holder. Insert the tubes with beads in the magnet holder. Wait until all are attached to the magnet. The solution will appear clear. Quickly aspirate off the buffer.

10. Repeat washing step with 0.1M Na Phosphate pH7.4 - incubation for 10 minutes is not necessary – Add 4ml of 0.1M NaPO4 to the Dynabeads in each tube. Vortex 15 seconds. Put on the Magnet- Aspirate off the buffer.

11. Add 3ml of AB mix to each tube. Vortex.

12. Transfer to four 15ml screw-caps Falcon tubes (these are better for rotating overnight because they don't leak). Rinse tubes again with a total of 2ml x 2 of AB mix.

13. Vortex beads in Falcon tubes. Wrap with Parafilm

14. Rotate on the wheel at 30^0 C overnight

15. **<u>Next Day- Washing the Dynabeads after Conjugation</u>**
    Do all washes as described above after transferring the suspensions to 5 ml snap-cap Falcon tubes and by inserting the tubes into the Magnet holder. You can aspirate the supernatant by using a Vacuum Aspirator.

16. Wash once with 3 ml of 100mM Glycine HCL pH2.5- approximately 3ml – Put it on and take it off as fast as possible.

17. Wash once with 3ml of 10mM Tris pH 8.8

18. Wash once with 3 ml of fresh 100mM Triethylamine – [Make the 100 mM Triethylamine (stored in the hood) stock by adding approximately 140ul to 10ml of DDH20] - Put it on and take it off as fast as possible.

19. Wash the coated beads – a total of 4x with-1x PBS-in 5ml falcon tubes-4 x 5 minutes

20. Wash once with PBS + 0.5% Triton X-100- 5 minutes

21. Wash once with PBS +0.5% Triton X-100. Let stand 15 minutes on rocker

22. Finally, resuspend all beads in a total of 2ml of 1x PBS + 0.02% NaN3

23. Store the coated beads at 4 ^{0}C