Conjugation of Dynabeads with Rabbit IgG

The conjugation protocol gives 2mL of densely antibody-coated Dynabeads (~160µg of rabbit IgG per mg of Dynabeads) with a final concentration of ~0.15µg of beads per µL of solution. The beads can be stored for several months at 4°C without loss of activity.

Note1: We use rabbit IgG -100mg (Sigma, Cat. No. 15006)

Note2: We use M270 Epoxy Dynabeads - 300mg (Invitrogen, Cat. No. 14302D)

Note3: All buffers used in this protocol are stored at RT.

Note4: If less than 300mg of Dynabeads are conjugated at a time, then the entire conjugation protocol has to be scaled accordingly, including the volume of AB mix. The final concentration of ammonium sulfate has to be of 1M in the AB mix.

Day One – Conjugation

1) Reconstitute the magnetic beads:
   a. Vortex-resuspend the entire vial of Dynabeads (2 x 10^{10} beads) in 16ml of 0.1M $NaPO_4$ buffer - pH 7.4.

   b. Divide equally amongst the four Falcon tubes. Wash any remaining beads in the glass vial with an additional 2ml of 0.1M $NaPO_4$ buffer.

   c. Shake bead suspension slowly for at least 10 minutes on a Nutator or rocking platform.

2) Aliquot the antibody stock solution and prepare the AB mix:

   a. Resuspend Rabbit IgG (100mg) in 8mL double distilled water. (IgG conc. ~12.5mg/mL). Aliquot into 1 mL fractions and store any unused IgG at -80°C.

   b. Prepare AB mix:
      i) Spin down 4mL of Rabbit IgG (10 min, 14Krpm, 4°C). Use the supernatant and discard pellet.

      ii) In a 50mL falcon tube, mix the following:
      (1) 3.525mL of IgG
      (2) 9.85mL 0.1M $NaPO_4$ buffer
      (3) 6.65mL 3M Ammonium Sulfate. Add slowly shaking the tube a bit.

      iii) Filter solution using a .22 µm Millex GP filter.

3) O/N conjugation:
   a. Magnetically recover the beads until the bead solution will appears clear. Aspirate the buffer off using a Vacuum Aspirator or a pipetaid. (Be careful not to aspirate off the beads too).

   b. Wash again in 4mL 0.1M $NaPO_4$ (vortex, 15 sec). Magneticcaly recover beads and remove the buffer.

   c. To each tube, add 5mL of AB mix (Vortex, 15sec). Seal tops with Parafilm.

   d. Incubate (18-24hrs, rotating wheel, 30°C)

Day Two - Wash the Dynabeads after Conjugation

Do all washes as described in the 15mL Falcon tubes. Mix by inversion until all beads are resuspended. You can aspirate the supernatant by using a Vacuum Aspirator or a pipetaid. (Be careful not to aspirate off the beads too).

1) 1x 3mL of 100mM Glycine, pH 2.5 (do not leave the beads in this buffer for too long).

2) 1x 3mL of 10mM Tris-HCl, pH8.8.

3) 1x 3mL of 100mM Triethylamine (Make fresh under the hood: 168µL of stock in 11.832mL double distilled water) (do not leave the beads in this buffer for too long).

4) 5x 3mL of 1XPBS, rocking for at least 5 minutes.

5) 1x 3mL of 1XPBS, 0.5% Triton X-100, rocking for at least 5 minutes.

6) 1x 3mL of 1XPBS, 0.5% Triton X-100, rocking for 15 minutes.

7) Finally, combine the beads and resuspend them in 2ml of 1x PBS + 4µL 10%Sodium Azide. Store the coated beads at 4 ^{0}C.

Required Solutions

0.1M Sodium Phosphate Buffer ($NaPO_4$) – pH 7.4

2.62g NaH_2PO_4 x H_2O (MW 137.99)
14.42g Na_2HPO_4 x $2H_2O$ (MW 177.99)

Dissolve in distilled water, adjust pH if necessary and adjust to 1 liter.

3M Ammonium Sulfate (stock solution)

39.6g $(NH_4)_2SO_4$ (MW 132.1)

Dissolve in 0.1M Sodium Phosphate Buffer (pH 7.4) and adjust to 100mL

Phosphate Buffered Saline (PBS) - pH 7.4

0.26g NaH_2PO_4 x H_2O (MW 137.99)
1.44g Na_2HPO_4 x $2H_2O$ (MW 177.99)
8.78g NaCl (MW 58.5)

Dissolve in 900mL distilled water, adjust pH if necessary and adjust to 1 liter.

PBS + 0.5% Triton X-100

Include 0.5% (w/v) Triton X-100 in 100 mL PBS solution

100mM Glycine HCl pH 2.5

10mM Tris pH 8.8

10% Sodium Azide (NaN_3) – Careful, refer to MSDS