

Conjugation of Dynabeads with Rabbit IgG

Coupling of Dynabeads with Rabbit IgG in order to produce magnetic beads which are capable of pulling out various Protein A tagged complexes.

*Note: We are using rabbit IgG -100mg - from Sigma – catalogue number 15006
Dynabeads are from Invitrogen - Dynabeads M270 Epoxy*

Day One – Preparing the Beads and Conjugation

- 1) Resuspend entire vial of Dynabeads (2×10^{10} beads) in 16ml of 0.1M NaPO₄ buffer - pH 7.4.
- 2) Vortex bottle 30 seconds.
- 3) Divide bead suspension into four 15 ml Falcon tubes (4mL of suspension in each tube).
- 4) Wash any remaining beads in the glass vial with an additional 2ml of 0.1M NaPO₄ buffer. Divide equally amongst the four Falcon tubes.
- 5) Shake bead suspension slowly for 10 minutes on a Nutator or rocking platform.
- 6) While bead suspension is on Nutator prepare the AB mix.
 - a. FIRST: Resuspend the entire bottle of Rabbit IgG (100mg) in 7ml double distilled H₂O – this will result in a concentration ~14 mg/mL. Aliquot into 1 mL fractions and store any unused IgG at -20°C.
 - b. Spin down 3525uL, of Rabbit IgG, in a table top centrifuge, for 10 mins, at 14K rpm, at 4°C. Save supernatant and discard pellet.
 - c. Prepare AB mix by adding the solutions to a 50mL falcon tube, in the order listed.
 - i) 3525uL of IgG (which was previously spun down) *If IgG concentration needs to be altered please see end of protocol for instructions on determining appropriate amounts of Sodium Phosphate and Ammonium Sulfate*
 - ii) 9.850mL 0.1M NaPO₄ buffer.
 - iii) 6.650mL 3M Ammonium Sulfate. Add slowly shaking the tube a bit.
 - iv) Filter solution using a .22 µm Millex GP filter
- 7) Place the Falcon tubes with the bead suspension onto a magnetic holder and wait until all beads are attached to the magnet (DynaL MPC-6 Magnetic Particle Concentrator Prod. No. 120.02). Bead solution will appear clear. Aspirate the buffer off (be careful not to aspirate off the beads too).
- 8) Wash again with 4mL 0.1M NaPO₄ - incubation for 10 minutes is not necessary. Vortex 15 seconds. Put on the Magnet - aspirate off the buffer.
- 9) Add 5ml of AB mix to each tube vortex to completely combine the AB mix and the beads.
- 10) Wrap tops of Falcon tubes with Parafilm and place on rotating wheel at 30 °C overnight (incubation must last at least 18 hours but no more than 24 hours).

Day Two - Washing the Dynabeads after Conjugation

Do all washes as described in the 15mL Falcon tubes. You can aspirate the supernatant by using a Vacuum Aspirator.

- 1) Wash once with 3mL of 100mM Glycine HCL pH2.5. Put it on and take it off as fast as possible.
- 2) Wash once with 3mL of 10mM Tris pH 8.8.
- 3) Wash once with 3mL of 100mM Triethylamine. (**Make fresh** 100 mM Triethylamine by adding 168ul stock to 11.156mL of DDH2O). Put it on and take it off as fast as possible.
- 4) Wash the coated beads with 1x PBS for 5 minutes – washes should be done on a rocker/nutator – repeat 4x.
- 5) Wash once with PBS + 0.5% Triton X-100 for 5 minutes.
- 6) Wash again with PBS +0.5% Triton X-100 for 15 minutes on rocker/nutator.
- 7) Finally, resuspend all beads in a total of 2ml of 1x PBS + 0.02% Sodium Azide.
- 8) Store the coated beads at 4 °C.

Change in IgG volume

Original total reaction volume of the IgG, Sodium Phosphate, and the Ammonium sulfate is 20mL. To determine the new volume of Sodium Phosphate subtract the amount of IgG, and Ammonium sulfate from the original total reaction mixture (20mL). This will leave you with the new volume of Sodium phosphate. *Note: Only two volumes are changing in this reaction; the volume of IgG and the volume of Sodium Phosphate. The amount of Ammonium Sulfate remains constant.*

Necessary Solutions**0.1M Sodium Phosphate Buffer (NaPO₄) – pH 7.4**

2.62g NaH₂PO₄ x H₂O (MW 137.99)
14.42g Na₂HPO₄ x 2H₂O (MW 177.99)

Dissolve in distilled water, adjust pH if necessary and adjust to 1 liter. This buffer is for pre-washing beads, do not add any protein, sugar, etc.

3M Ammonium Sulfate (stock solution)

39.6g (NH₄)₂SO₄ (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 NaH₂PO₄ x H₂O (MW 137.99)
1.44g Na₂HPO₄ x 2H₂O (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 (NaN₃)**