Rabbit IgG Conjulation to Dynabeads

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[Abstract] This method couples rabbit immunoglobulin G (IgG) (or any other proteins to serve as affinity reagent) to the surface of magnetic beads (Dynabeads). The amine and thiol groups of amino acid residues on the protein are covalently linked with the epoxy group on Dynabeads. The coupled IgG beads can be stored at 4 °C for 6-12 months with no obvious loss in reactivity.

Materials and Reagents

1. Invitrogen Conjulation kit (143.11D), contains C1, C2, HB, LB, SB solutions
2. IgG (Sigma-Aldrich, catalog number: I5006-10 mg)
3. Dynabeads M-270 Epoxy 300 mg (Life Technologies, Invitrogen™)
4. Phosphate buffered saline (PBS) tablet
5. 30% NaN_3 (fresh)
6. 20% Tween 20

Equipment

1. Hula mixer (Life Technologies, Invitrogen™) or other rocker capable of 360° rotation
2. Magnet for 15 ml tubes
3. Spectrometer

Procedure

Day 1

1. Dissolve rabbit IgG in cold 1x PBS to a final concentration of 10 mg/ml.
2. Centrifuge the rabbit IgG at 14,000 rpm for 10 min at 4 °C, and save the supernatant.
3. Determine the IgG concentration using the Bradford method on a spectrometer.
4. Add 5 ml C1 of the Conjulation Kit to the 300 mg dynabeads and mix by pipetting in the original glass vial. Then transfer to a 15 ml tube. Add C1 to 12 ml, split to another three 15 ml tubes, each tube with 3 ml beads.
5. Remove the supernatant from the tube on a magnet.

6. Add 300 µl rabbit IgG from step 1, 2.7 ml C1 to the beads, mix by pipetting.
7. Add 3 ml C2 and mix by pipetting again.
8. Wrap in aluminum foil and incubate at 37 °C for 16-24 h on Hula mixer set at 15-20 rpm.

Day 2

1. Centrifuge 1,000 *x g*, 5 min. Remove the supernatant (save supernatant for calculation of protein conjulation efficiency).
2. Wash each tube with 1,000 µl HB in each tube (add HB, then transfer to a 2 ml tube). Combine supernatant.
3. Wash each tube with 1,000 µl LB. Combine supernatant.
4. Wash each tube with 1,000 µl SB. Combine supernatant.
5. Wash each tube with 1,000 µl SB and incubate at room temperature (RT) for 15 min. Combine supernatant.
6. Wash each tube with 1,000 µl PBS+0.05% Tween at RT for 10 min x 3 times.
7. Resuspend beads in each tube with 2 ml SB, add NaN_3 to final concentration of 0.02%. Then split each tube of beads (400 µl) to 5 tubes containing 600 µl SB each with 0.02% NaN_3.

References

1. Li, X., Gianoulis, T. A., Yip, K. Y., Gerstein, M. and Snyder, M. (2010). Extensive *in vivo* metabolite-protein interactions revealed by large-scale systematic analyses. *Cell* 143(4): 639-650.

