11/03/2023 Version – John Blair, adapted from Promega

Antibody Cleanup for Conjugation Prep

In order to use the protocol you must purchase Promega Magne Protein G beads (cat#:G7471). This protocol will take you through the steps to clean up your antibody of interest from proteins that will affect conjugation including BSA and Ascites solution. If your protein is stored in PBS or PBS with glycerol with no carrier proteins, it is not necessary to perform this cleanup and you can proceed directly to the conjugation.

Materials to Be Supplied by the User

Bind/wash buffer – 1X PBS

Elution buffer – 100 mM Glycine-HCl-pH 2.7

- 0.375g Glycine
- Dissolve in 40 ml H2O
- Adjust to pH 2.7 with HCl
- Bring volume to 50 ml

Neutralization buffer – 2M Tris buffer pH 7.5

Magnetic stand for 1.5 ml tubes

Tube rotator

Procedure

Note: All volumes below can be scaled up as necessary

1. Gently vortex or invert the beads to obtain a uniform suspension. Keep the suspension uniform when aliquoting beads.
2. Add 25µl of bead slurry to a 1.5ml tube. Place in the magnetic stand for 10 seconds.
3. Remove and discard the storage buffer.
4. Add 250µl of bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard the bind/wash buffer.
5. Combine 25µl of bind/wash buffer and 25µl of sample, then add to the equilibrated beads.
6. Mix sample for 30–60 minutes at room temperature. Make sure the beads remain in suspension.
7. Place tube in the magnetic stand for 10 seconds. Remove the supernatant, and save for running on a gel at the end.
8. Wash beads by adding 250µl of bind/wash buffer and mix for 5 minutes. Place in the magnetic stand for 10 seconds. Remove and discard bind/wash buffer.
9. Repeat step 8 for a total of two washes.
10. Wash beads by adding 100µl of bind/wash buffer. Mix and place in the magnetic stand for 10 seconds. Remove and discard all bind/wash buffer.
11. Add 25µl of elution buffer [100mM glycine-HCl (pH 2.7)] to the beads.
12. Mix for 5 minutes at room temperature.

13. Place tube in the magnetic stand for 10 seconds. Remove eluted sample, and transfer to a new microcentrifuge tube containing 5µl of neutralization buffer [2M Tris buffer (pH 7.5)]. This is the first elution.
14. Repeat elution Steps 11–13 into the same tube.
15. Quantify the antibodies using a BCA assay. Run a small fraction (1ug) of the purified sample and the supernatant side by side on a gel to verify the complete removal of carrier proteins.