Mouse hybridoma antibody purification using pG-Dynabeads

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This protocol is adapted from NEB & pG-Dynabead antibody purification protocols. For pA-Dynabeads the elution pH will differ slightly. Refer to the pA-Dynabead literature for these details.

Bind antibody to beads:

1. Vortex & resuspend pG-Dynabeads
2. 25 µL Beads to 1.5 mL Eppendorf
3. Add 500 µL **Binding Buffer** (Sodium Phosphate [0.1M], pH = 8.0)
   - Vortex to resuspend
   - Pop spin ≤200g to collect beads from cap
   - Bind to magnet, remove supernatant
   - Repeat 1x
4. Add 80 µL **Binding buffer**, and 15-25 µL **antibody**
   - Spin antibody 1'x2500g to pellet any nuclei before addition
5. Mix & Incubate RT x 40' w/ rocking
6. Pop spin, bind to magnet, remove sup
   - Wash 3x 500 µL **Binding Buffer** (vortex to resuspend, 1' w/ rocking @ RT, spin, repeat)

Elute Antibody:

1. Prepare 2x 1.5 mL tubes w/ 10 µL **Tris-HCl** ([1M], pH = 9.0)
2. Add 50 µL **Glycine** ([0.2 M], pH = 2.5) to beads from step 6. above
   - Vortex
   - Tilt rotate x2'
3. Pop spin, bind to magnet
   - Transfer sup to Tris-HCl tube to neutralize
   - **NOTE:** You'll always transfer a little bit of bead dust at this step. Therefore, before using the purified antibody, bind it to a magnet to minimize transfer of bead-bound material.
4. Repeat steps 2&3
   - Pool eluates
   - Store purified supernatant at 4°C

Quality Test for Purified Antibody:

1. Perform Immunofluorescence using purified & unpurified antibody
2. Qubit 1 µL of purified & unpurified antibody
   - DNA should be undetectable in purified sample

Reagents:

Binding Buffer – 0.1 M Sodium Phosphate pH 8.0

- Make by mixing 9.32 mL Na_2HPO_4 [1M] + 0.68 mL NaH_2PO_4 [1M] + 90 mL dH_2O

Tris-HCl – 1M pH 9.0

Glycine – 0.2M pH 2.5