LOABeads Protein A – Quick Protocol

For all separation steps, use the LOABeads MagSep Cube or LOABeads MagSep 15/50 magnets. Only remove liquid when beads are fully separated by magnet.

Purification of 1–2 mg IgG from serum or ascites

1. Dispense 500 µl well-mixed bead suspension into a test tube.
2. Wash 1x with 500 µl PBS.
3. Add 500 µl PBS + 250–500 µl serum or ascites.
4. Mix for 30–60 min.
5. Wash at least 3x with 500 µl PBS.
6. Add 300–500 µl 60 mM citrate, pH 3.0.
7. Mix for 1 min to elute.
8. Add 30–50 µl 2 M Tris-HCl, pH 9.0, to the saved supernatant for neutralization.
9. Elute a 2nd time if needed.
10. Regenerate beads and store in PBS, 20% EtOH.

Depletion of IgG from serum

1. Perform steps 1–4 above.
2. Save the supernatant, which represents the IgG-depleted serum.
3. Regenerate beads and store in PBS, 20% EtOH.

Immunoprecipitations

- Use 50–200 µl 10% LOABeads Protein A suspension (5–20 µl settled beads) per immunoprecipitation.
- Perform all steps throughout the immunoprecipitation as with classical, non-magnetic protein A agarose, except use the LOABeads MagSep Cube magnet for separations.