Protocol for removal of BSA from antibodies

BSA depletion agarose facilitates fast & easy removal of BSA from antibodies with high antibody recovery. BSA is often added to antibodies for stabilization but may interfere with conjugation methods and other downstream applications.

General Notes

- BSA Depletion Agarose is a high-affinity binding resin binding BSA to deplete it from an antibody solution. The antibody of interest will flow through.
- BSA Depletion Agarose is compatible with antibodies from a wide range of species such as rabbit, mouse, rat, chicken, sheep etc.
- BSA Depletion Agarose is also compatible with typical antibody buffer compounds, including glycerol up to 50%.
- BSA concentrations in antibodies can vary from 0.01% to 1%.
  Our recommendation for usage:

    - Use 50 µl **BSA Depletion Agarose** (slurry) for 100 µl of antibody supplemented with ≤ 0.1% BSA.
      Note: *We do not recommend using less than 50 µl to avoid significant loss of antibody due to bead handling issues.*
    - Use 200 µl beads (slurry) for 100 µl antibody solutions with 0.5% BSA.
    - Use 300 µl beads (slurry) for 100 µl antibody solutions with 1% BSA.

  Bead volumes can be scaled up proportionally to the antibody volume.

- Important note: Commercial BSA preparations often contain bovine serum proteins, including bovine immunoglobulins (IgG). As a result, the recovered IgG may appear lower in quantity, when in fact the purification has removed contaminating bovine IgG and yielded a cleaner preparation of the target IgG.

Procedure

Equilibration of beads (for 100 µl antibody volume)

1. Resuspend beads by pipetting up and down or by inverting. Do not vortex.
2. Remove stopper from a spin column and insert it into a 2 ml Eppendorf tube.
3. Transfer 100 µl (for BSA concentrations < 0.5%) or 300 µl (for BSA 1%) beads (slurry) to a spin column. Centrifuge at 2000x g for 1 min to remove storage solution.
4. Add 500 µl PBS to the beads and centrifuge at 2000x g for 1 min. Discard the flow-through.

Depletion of BSA

5. Close the spin column and add your antibody (100 µl) to the equilibrated beads.
6. Incubate for 15 min at room temperature at 1000 rpm in a thermal shaker.
7. Open spin column and transfer to fresh 2 ml Eppendorf tube.
8. Centrifuge at 2000x g for 2 min to recover antibody.
9. Optional (may increase antibody yield): Add 100 µl PBS to the beads and centrifuge at 2000x g for 2 min. Combine eluate with eluate from step 8.

Note: This will decrease the concentration of the antibody.