Protocol for removal of excess conjugate from conjugated primary antibodies

Conjugation CleanAb Kits (Magnetic Agarose) facilitate fast & easy clean-up of conjugated rabbit IgG (Cat no. CK002) or mouse IgG (Cat no. CK004) to remove excess conjugate at a native pH (pH 7 - 7.5) and with a high recovery rate.

General Notes

- Conjugation CleanAb Kits are compatible with antibodies conjugated via common techniques such as NHS ester – amine reaction, maleimide – thiol reaction, or click-chemistry.
- All components needed for the protocol are supplied with the kit, except for Eppendorf tubes.
- 100 µl of magnetic beads (slurry) can be used for purification of ~ 150 µg of conjugated primary antibody. Bead volumes can be adjusted proportionally to fit your experimental needs.

There are two ways to use CleanAb:

1. **Post conjugation clean-up**
   Conjugate the IgG in solution using your preferred technique and use the CleanAb kit to remove free conjugate afterwards.

2. **On-bead conjugation and clean-up**
   Immobilize the IgG on CleanAb beads, wash away any undesired buffer components, and perform your conjugation on the beads. This method removes the need for buffer exchange prior to conjugation to remove incompatible components (e.g., Tris, BSA).

Option 1: Post conjugation clean-up (removal of excess conjugate)

Procedure

Equilibration of beads

1. Resuspend the beads by gently pipetting or by inverting the tube. Do not vortex.
2. Transfer 100 µl beads (slurry) to a 1.5 mL tube.
3. Add 500 µl Wash buffer to the beads and separate with magnet (or centrifuge at 2000x g for 1 min). Discard the supernatant.

Binding of the conjugated IgG

4. Add your conjugated primary rabbit or mouse IgG (up to 150 µg) to the equilibrated beads. Add wash buffer to a final volume of 100 µl if input volume is below that.
5. Incubate for 30 min at room temperature under end-over-end rotation (or at 1000 rpm in thermal shaker if sample volume is <200 µL). Make sure beads do not settle.

Washing of beads

6. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
7. Resuspend beads in 500 µL Wash buffer.
8. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
9. Repeat steps 7 - 8 twice.

Elution of the conjugated IgG

10. Add 100 µl Elution buffer (pre-equilibrated to room temperature) to beads.
11. Incubate beads at room temperature for 10 min while mixing at 1000 rpm in thermal shaker. Make sure beads do not settle.
12. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min). Save the eluate, which will contain the purified IgG-conjugate.
13. Repeat steps 10 - 12.
14. Pool eluates.

Optional: Buffer exchange to remove Elution buffer

Use a Zeba spin desalting column or similar and follow the manufacturer's instructions and use your desired buffer for long term storage.

Note: The Elution buffer contains high concentrations of certain reagents that might affect running behavior in SDS PAGE.

Exchanging the buffer is recommended for long-term storage at -20°C (> 6 months). Storage at -80°C does not require buffer exchange.

Please note that a buffer exchange can lead to minor sample loss.

Option 2: On-bead conjugation and clean-up

Procedure

Equilibration of beads

1. Resuspend beads by pipetting up and down or by inverting. Do not vortex.
2. Transfer 100 µl beads (slurry) to a 1,5 mL tube.
3. Add 500 µl Wash buffer to the beads and separate with magnet (or centrifuge at 2000x g for 1 min). Discard the supernatant.

Binding of the conjugated IgG

4. Add your conjugated primary rabbit or mouse IgG (up to 150 µg) to the equilibrated beads. Add wash buffer to a final volume of 100 µl if input volume is below that.
5. Incubate for 30 min at room temperature under end-over-end rotation (or at 1000 rpm in thermal shaker if sample volume is <200 µL). Make sure beads do not settle.

Washing of beads

6. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
7. Resuspend beads in 500 µL of a buffer suitable for your conjugation technique.
8. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
9. Repeat steps 8 - 9 twice.

On-bead conjugation

10. Perform on-bead conjugation using the appropriate buffer and reagents for your conjugation technique of choice. Use similar amounts as you would have used for a conjugation in solution or slightly more for NHS conjugation. Incubate under end-over-end rotation (or at 1000 rpm in thermal shaker if sample volume is <200 µL). Make sure beads do not settle.

    Note: If you use a two-step conjugation strategy (e.g., first introducing a click-chemistry handle, then the addition of the desired molecule using click-chemistry), return to step 6. Otherwise, carry on with step 11.

11. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
12. Resuspend beads in 500 µL Wash Buffer.
13. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Discard supernatant.
14. Repeat steps 12 - 13 twice.

Elution of the conjugated IgG

15. Add 100 µl Elution buffer (pre-equilibrated to room temperature) to beads.
16. Incubate beads at room temperature for 10 min while mixing at 1000 rpm in thermal shaker. Make sure beads do not settle.
17. Separate beads with magnet (or centrifuge the spin column at 2000x g for 1 min.) Save the eluate, which will contain the purified IgG-conjugate.
18. Repeat steps 15–17.
19. Pool eluates.

Optional step: Buffer exchange to remove Elution buffer

20. Use a Zeba spin desalting column or similar and follow the manufacturer's instructions and use your desired buffer for long term storage.

Note: The Elution buffer contains high concentrations of certain reagents that might affect running behavior in SDS PAGE and concentration determination. If you need to measure concentrations precisely, we recommend doing the buffer exchange step.

For long-term storage at -20°C (> 6 months), buffer exchange is also recommended. Storage at -80°C does not require buffer exchange.

Please note that a buffer exchange can lead to minor sample loss.