Protocol for removal of excess oligonucleotide from oligo-conjugated primary antibodies

Conjugation CleanAb Kits facilitate fast & easy clean-up of conjugated rabbit IgG (Cat no. CK001) or mouse IgG (Cat no. CK003) to remove excess oligonucleotide at near native pH (pH 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 Beads (slurry) can be used for purification of up to 150 µg of conjugated primary antibody. Bead volumes can be adjusted proportionally to fit your experimental needs.
- The following protocol describes the use of 100 µl of CleanAb Beads (slurry).

- 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 oligos 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 them up and down or by inverting the tube. Do not vortex.
2. Remove stopper from a spin column (provided with the kit) and insert it into a 2 ml Eppendorf tube.
3. Transfer 100 µl beads (slurry) to a spin column.
4. Add 500 µl Wash buffer to the beads and centrifuge at 2000x g for 1 min. Discard the flow-through.

Binding of IgG-oligonucleotide

5. Close the spin column and 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.
6. Incubate for 30 min at room temperature under end-over-end rotation.

Washing of beads

7. Open spin column and insert into a 2 ml Eppendorf tube.
8. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
9. Resuspend beads in 500 µL Wash buffer.
10. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
11. Repeat steps 9–10 twice.

Elution of IgG-oligonucleotide

12. Close spin column and add 100 µl Elution buffer (pre-equilibrated to room temperature) to beads.
13. Incubate beads at room temperature for 10 min under end-over-end rotation.
14. Open spin column and transfer to fresh 2 ml Eppendorf tube.
15. Centrifuge the spin column at 2000x g for 2 min.
    Save the eluate, which will contain the purified IgG-oligonucleotide conjugate.
16. Repeat steps 12–15.
17. Pool eluates.

Optional: Buffer exchange to remove Elution buffer

1. 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.

Option 2: On-bead conjugation and clean-up Procedure

Equilibration of beads

2. Resuspend the beads by gently pipetting them up and down or by inverting the tube. Do not vortex.
3. Remove stopper from a spin column (provided with the kit) and insert it into a 2 ml Eppendorf tube.
4. Transfer 100 µl beads (slurry) to a spin column.
5. Add 500 µl Wash buffer to the beads and centrifuge at 2000x g for 1 min. Discard the flow-through.

Binding of IgG-oligonucleotide

6. Close the spin column and add your 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.
7. Incubate for 30 min at room temperature under end-over-end rotation.

Washing of beads

8. Open spin column and insert into a 2 ml Eppendorf tube.
9. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
10. Resuspend beads in 500 µL of a buffer appropriate for your conjugation technique.
11. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
12. Repeat steps 9–10 twice.

On-bead conjugation

13. Close the spin column.

    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.

    Note: If you use a two-step conjugation strategy (e.g., introduction of a click-chemistry handle in the first step, then the addition of the oligonucleotide using click-chemistry in the second step), return to step 7. Otherwise, carry on with step 14.

14. Open spin column and insert into a 2 ml Eppendorf tube.
15. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
16. Resuspend beads in 500 µL Wash buffer.
17. Centrifuge the spin column at 2000x g for 1 min. Discard flow-through.
18. Repeat steps 16–17 twice.

Elution of IgG-oligonucleotide

19. Close spin column and add 100 µl Elution buffer (pre-equilibrated to room temperature) to beads.
20. Incubate beads at room temperature for 10 min under end-over-end rotation.
21. Open spin column and transfer to fresh 2 ml Eppendorf tube.
22. Centrifuge the spin column at 2000x g for 2 min.

    Save the eluate, which will contain the purified IgG-oligonucleotide conjugate.
23. Repeat steps 19–22.
24. Pool eluates.

Optional step: Buffer exchange to remove Elution buffer

25. 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.