Procedure for crosslinking the antibody to the beads

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Reducing the amount of antibody contamination in eluted protein solution

To enable elution of protein with little antibody contamination (for cleaner protein preparation and cleaner western blots), it is recommended to cross link the antibody to the beads. An example procedure for this is shown below (there is much information on this procedure on internet websites). The target protein should then be eluted with a mild eluent, such as glycine buffer.

Reagents

Cross linking reagent:
Dimethyl pimelimidate (DMP)
Stock concentration 13 mg/ml DMP.

Elution reagent:
1 M glycine (Add conc. HCl to correct pH to pH3)

Dilution buffer:
PBS + 1 mg/ml BSA

Wash buffer:
0.2 M triethanolamine in PBS (3.04 ml triethanolamine per 100 ml buffer)

Quenching buffer:
50 mM ethanolamine in PBS (311.7 ul per 100 ml)

Preparation

1. Wash beads twice in PBS.

The end concentration should be 50% bead slurry.

2. Mix well and rotate overnight at 4°C.

Cross-linking

1. Wash the beads by centrifuging (14,000 rpm, 1 min) into a pellet. Aspirate out the PBS supernatant.
2. Add dilution buffer at 1:1 ratio, mix gently and rotate for 10 minutes at 4°C. Centrifuge and aspirate/discard the supernatant as before.
3. Prepare the antibody solution in Dilution buffer at the required concentration (see antibody datasheet for suggested concentration). Add diluted antibody at 1:1 ratio to the beads. Mix gently and rotate 1 hr at 4°C.
4. Centrifuge and aspirate/discard the supernatant.
5. Add dilution buffer to beads at 1:1 ratio. Rotate for 5 min at 4°C. Centrifuge and aspirate/discard the supernatant.
6. Add PBS to beads at 1:1 ratio. Centrifuge and aspirate/discard the supernatant.
7. Cross-linking:

DMP is unstable in aqueous solution. Prepare solution immediately prior to use.

Dissolve 1ml of prepared 13mg/ml stock of DMP with 1 ml Wash buffer. Vortex immediately to mix.
Add DMP solution to beads at 1:1 ratio. Rotate for 30 min at room temperature.

N/B You will need to verify pH of DMP is between 8-9 before and after addition to beads (cross-linking efficiency is greatly reduced outside this pH range).

Wash the beads with Wash buffer (rotate 5 min RT, then spin and aspirate).
Add DMP for second time at 1:1 ratio, rotate 30 min RT, wash as before.
Add DMP for third time at 1:1 ratio, rotate 30 min RT, wash as before.

8. Quench and wash.
   Add Quench buffer at 1:1 ratio, rotate 5 min RT, spin and aspirate; repeat.
   Wash with PBS.

9. Remove excess (unlinked) Antibody:
   Wash with 1 M glycine pH 3. Rotate 10 min RT. Repeat.

10. Storage washes.
    Wash with buffer to be used for immunoprecipitation (usually PBS+TWEEN). Rotate 5 min RT.
    Wash three times and store in final wash (after rotation). Beads can be stored at 4oC for a few days. Sodium azide can be added to prevent bacterial growth.

Immunoprecipitation

The antibody bound beads can now be used in a normal IP procedure, as above.
Elution of bound antigens:
To prevent elution of antibody with the target protein, use a gentle glycine elution gradient (up to 1 M).