Cold Spring Harbor Protocols

# Conjugation of Magnetic Beads for Immunopurification of Protein Complexes

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Protocol

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## INTRODUCTION

Knowledge of the composition of protein complexes provides key insights into their functions. Immunoaffinity purification provides an effective means for isolating protein complexes and elucidating their composition. Immunoisolation is achieved with antibodies directed either specifically against the proteins of interest or against tags that are coupled to the proteins of interest. This approach uses immunoaffinity purification on magnetic beads coated with antibodies for the rapid and efficient purification of protein complexes from cells or tissues. This protocol describes conjugation of magnetic beads with antibodies in preparation for immunopreciptation of protein complexes.

## RELATED INFORMATION

A protocol is available for **Affinity Purification of Protein Complexes** (Cristea and Chait 2011).

This method was originally developed using a green fluorescent protein (GFP) tag for the consecutive visualization and isolation of protein complexes in living systems (Cristea et al. 2005). This methodology has been used successfully to look for interactions in both yeast and mammalian systems, such as studies of the dynamic virus–host protein interactions during the course of a viral infection (Cristea et al. 2006).

Three critical concepts are important in performing this protocol. First, work quickly to minimize the dissociation of endogenous complexes and to limit the formation of nonphysiological interactions. Second, work at 4°C and keep solutions on ice to limit unwanted proteolysis and degradation. Third, minimize diluting protein extracts to minimize the dissociation of native complexes and formation of nonspecific interactions. Most protein complexes utilize noncovalent forces to maintain the interactions.

## MATERIALS

**RECIPES**: Please see the end of this article for recipes indicated by <**R**>.

**It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.**

### Reagents

Ammonium sulfate (3 M)
Anti-green fluorescent protein (GFP) antibodies
Glycine-HCl (100 mM, pH 2.5)
Immunoglobulin G (IgG) or high-affinity purified antibodies
Phosphate-buffered saline (PBS)
PBS, 0.02% sodium azide ($NaN_3$)
PBS, 0.5% Triton X-100

Adapted from *Proteomics: A Cold Spring Harbor Laboratory Course Manual* (ed. Link and LaBaer). CSHL Press, Cold Spring Harbor, NY, USA, 2009.
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<R>Sodium phosphate buffer (0.1 M, pH 7.4)
Triethylamine (100 mM)
*Prepare the triethylamine solution fresh just before use in Step 6.*
Tris-HCl (10 mM, pH 8.8)

### Equipment

Dynabeads, M-270 Epoxy (Dynal/Invitrogen)
Magnetic particle concentrator, 1.5-mL tube type (Dynal/Invitrogen 12020D)
Microcentrifuge tubes, round-bottomed
Micropipettor and tips
Neodymium magnets (Dynal/Invitrogen or National Imports MAGCRAFT)
*Rare-earth neodymium magnets are used to capture the Dynabeads. Dynal/Invitrogen offers different-sized magnetic particle concentrators. In addition, National Imports offers a large variety of economical MAGCRAFT neodymium magnets in different shapes and sizes that can be used to capture Dynabeads. The magnets can be temporarily attached to the sides of tubes using rubber bands.*
Rotating wheel in a 30°C environment
Tube shaker (e.g., MT-360 Microtube Mixer; Tomy)

## METHOD

*It is best to carry out Steps 1–5 in the afternoon (~4:00 PM) and wash the conjugated beads the next morning.*

1. Weigh the needed amount of magnetic beads in a round-bottomed microcentrifuge tube.

   *Isolations using 1 mg of beads are good for small-scale pilot experiments; using 4 mg of beads is usually sufficient for performing a single isolation, but this amount will depend on the abundance of the protein of interest. Using 10–20 mg of beads is appropriate when isolating highly abundant proteins.*

2. Wash the beads with 1 mL of 0.1 M sodium phosphate buffer (pH 7.4), vortex for 30 sec, and mix for 15 min on a tube shaker at room temperature.

3. Place the tube with the bead slurry on the magnet, remove the buffer, and wash the beads again with 1 mL of 0.1 M sodium phosphate buffer (pH 7.4). Vortex for 30 sec, place the tube on the magnet, and remove the buffer.

4. Resuspend the beads with IgG, anti-GFP antibodies, or other antibodies that are to be used for the affinity isolations. Use ~20 µL total volume per milligram of beads.

   *Carefully calculate the amount of antibody and solution volumes to use for each experiment. Use 10 µg of Ab/mg beads for IgG and commercially available antibodies, and 5 µg of Ab/mg beads for purified, high-affinity, custom-made antibodies. Saturation of 1 mg of M-270 beads is achieved with ~7–8 µg of antibody or IgG (Fig. 1). Using more than these specified amounts will lead to an unacceptable background from unbound antibody.*

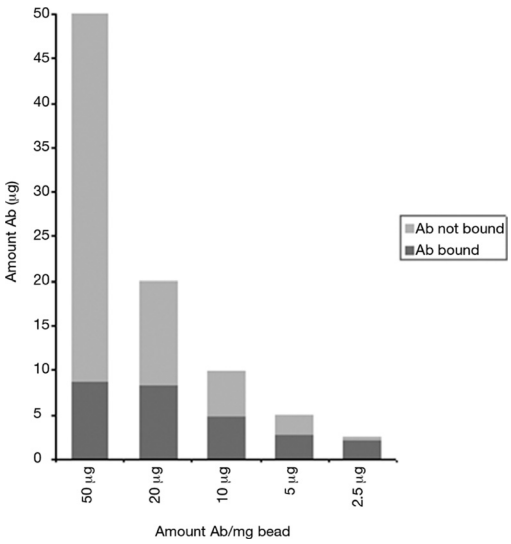


**FIGURE 1.** Binding capacity of magnetic affinity capture beads for antibodies. The graph shows that maximum binding is achieved with 7–8 µg of antibodies per mg of magnetic beads. (Reprinted, with permission, from the American Society for Biochemistry and Molecular Biology, Inc.)

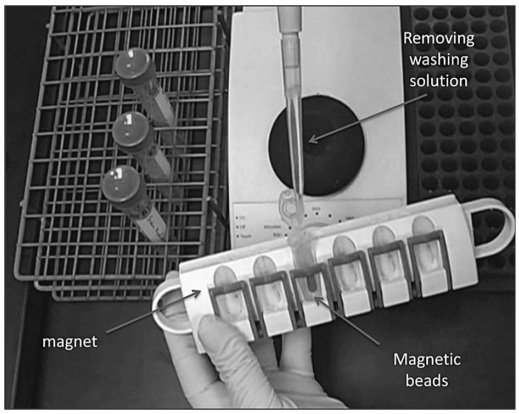


**FIGURE 2.** Using a magnetic concentrator to capture the magnetic beads for washing. The magnetic beads are held against the side of the tube by the magnetic particle concentrator while the liquid is carefully removed using a clean pipette tip.

*Here, we give an example in which antibodies are conjugated to 10 mg of beads. Prepare the reaction mix as per the table. It is important to add the components in the order given in the first column. Add the 3 M ammonium sulfate last to a final concentration of 1 M.*

| Reagent | Volume | Example: To conjugate IgG to 10 mg of beads, then total reaction volume is ~200 µL |
|---|---|---|
| Magnetic beads | | 10 mg |
| Antibody solution IgG | $V_{Ab}$ = volume to achieve the desired Ab concentration (see notes above) | e.g., volume to achieve final IgG amount of 100 µg |
| <**R**>Sodium phosphate buffer (0.1 M, pH 7.4) | $V_T - V_{Ab} - V_{sulf}$ | 200 µL – $V_{Ab}$ – 66.67 µL |
| Ammonium sulfate (3 M) | $V_{sulf}$ = 33% of $V_T$ for a final concentration of 1 M | 66.67 µL |

$V_T$ = total volume; $V_{Ab}$ = antibody solution volume; $V_{sulf}$ = 3 M ammonium sulfate volume.

5. Conjugate the antibodies to the beads overnight on a rotating wheel at 30°C.

6. In the morning, place the tube on a magnet. Remove the supernatant and wash the beads sequentially with:
   1 mL of 0.1 M sodium phosphate buffer (pH 7.4)
   1 mL of 100 mM glycine·HCl (fast wash)
   1 mL of 10 mM Tris-HCl (pH 8.8)
   1 mL of 100 mM triethylamine (freshly prepared; fast wash)
   Four washes of 1 mL each with PBS
   1 mL of PBS containing 0.5% Triton X-100 for 15 min
   1 mL of PBS

   *Be careful not to lose magnetic beads during the washes. After each washing step, the washing solution should have a clear aspect, with no trace of beads (Fig. 2).*

7. Store the beads at 4°C in PBS, 0.02% $NaN_3$.

   *Beads should be used within 2–3 wk of conjugation. After 1 mo of storage, their isolation efficiency decreases by ~40%.*

## REFERENCES

Cristea IM, Chait BT. 2011. Affinity purification of protein complexes. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5611.

Cristea IM, Williams R, Chait BT, Rout MP 2005. Fluorescent proteins as proteomic probes. *Mol Cell Proteomics* **4:** 1933–1941.

Cristea IM, Carroll JW, Rout MP, Rice CM, Chait BT, MacDonald MR 2006. Tracking and elucidating alphavirus–host protein interactions. *J Biol Chem* **281:** 30269–30278.

## RECIPES

### Sodium phosphate

1 M sodium phosphate buffer (pH 6.0–7.2)
Mixing 1 M $NaH_2PO_4$ (monobasic) and 1 M $Na_2HPO_4$ (dibasic) stock solutions in the volumes designated in the table below results in 1 L of 1 M sodium phosphate buffer of the desired pH. To prepare the stock solutions, dissolve 138 g of $Na_2HPO_4 \cdot H_2O$ (monobasic; m.w. = 138 g) in sufficient $H_2O$ to make a final volume of 1 L and dissolve 142 g of $Na_2HPO_4$ (dibasic; m.w. = 142 g) in sufficient $H_2O$ to make a final volume of 1 L.

| Volume (mL) of 1 M $NaH_2PO_4$ | Volume (mL) of 1 M $Na_2HPO_4$ | Final pH |
|---|---|---|
| 877 | 123 | 6.0 |
| 850 | 150 | 6.1 |
| 815 | 185 | 6.2 |
| 775 | 225 | 6.3 |
| 735 | 255 | 6.4 |
| 685 | 315 | 6.5 |
| 625 | 375 | 6.6 |
| 510 | 490 | 6.8 |
| 450 | 550 | 6.9 |
| 390 | 610 | 7.0 |
| 330 | 670 | 7.1 |
| 280 | 720 | 7.2 |

0.1 M sodium phosphate buffer (pH 7.4)
Add 3.1 g of $NaH_2PO_4 \cdot H_2O$ and 10.9 g of $Na_2HPO_4$ (anhydrous) to distilled $H_2O$ to make a volume of 1 L. The pH of the final solution will be 7.4. This buffer can be stored for up to 1 mo at 4°C.

0.1 M sodium phosphate buffer (from 1 M stocks) at 25°C

To prepare 1 L of 0.1 M sodium phosphate buffer of the desired pH, the following mixtures should be diluted to 1 L (final volume) with $H_2O$.

| pH | Volume (mL) of 1 M $Na_2HPO_4$ | Volume (mL) of 1 M $NaH_2PO_4$ |
|---|---|---|
| 5.8 | 7.9 | 92.1 |
| 6.0 | 12.0 | 88.0 |
| 6.2 | 17.8 | 88.2 |
| 6.4 | 25.5 | 74.5 |
| 6.6 | 35.2 | 64.8 |
| 6.8 | 46.3 | 53.7 |
| 7.0 | 57.7 | 42.3 |
| 7.2 | 68.4 | 31.6 |
| 7.4 | 77.4 | 22.6 |
| 7.6 | 84.5 | 15.5 |
| 7.8 | 89.6 | 10.4 |
| 8.0 | 93.2 | 6.8 |