

LAB 6 Purification and Storage of Antibodies

Purification of Antibody

1. Human serum diluted in a proportion of 1:2 with saline
2. A saturated ammonium sulphate solution (prepared by dissolving 20 g of ammonium sulphate in 20 ml of PBS at 50°C and the solution stored for overnight at 4°C) was added slowly to immunoglobulin solution with continuous agitation to a final concentration 45% saturation (v/v).
3. The contents of the tube stirred at room temperature for 30 min. then centrifuge 3000g for 15 min at 4°C
4. The precipitate then washed 3 times with 45% saturated ammonium sulphate.
5. The precipitate re-dissolved in the same volume of PBS as the original serum volume and centrifuged to remove any insoluble materials and dialyzed against 5 changes of PBS at 4°C.

Guidelines for the storage of different types of antibody

In comparison to sensitive proteins such as enzymes, antibodies are highly stable proteins that retain activity in a wide range of biological conditions. As a result, storage is reasonably straight forward and should focus on two key considerations:

- 1) Antibodies are sensitive to repeated freezing/thawing cycles
- 2) Excessive protein concentration dilution (particularly of purified antibody) can result in material losses and reductions in antibody stability.

Storage temperatures and conditions

For many of our antibodies, freezing at -20°C or -80°C in small aliquots is the optimal storage condition **because** aliquoting minimizes damage due to freezing and thawing, as well as contamination introduced by pipetting from a single vial multiple times.

The size of the aliquots will depend on how much one typically uses in an experiment. Aliquots should be no smaller **than 10 µl**; the smaller the aliquot, the more the stock concentration is affected by evaporation and adsorption of the antibody onto the surface of the storage vial.

Storage at 4°C upon receipt of the antibody is acceptable for **one to two weeks**, followed by freezing for long-term storage.

Preventing contamination with sodium azide

To prevent microbial contamination, **Sodium Azide** can be added to an antibody preparation to a final concentration of 0.02% (w/v). Many antibodies already contain this preservative at concentrations ranging from 0.02 to 0.05%.

When NOT to use sodium azide:

1. If staining or treating live cells with antibodies,
2. if using antibodies for *in vivo* studies

be sure to use preparations that **do not contain sodium azide**. This antimicrobial agent is toxic to most other organisms. Sodium azide can be removed from antibody solutions by **dialysis or gel filtration**

Freezing /thawing damage

Repeated freezing/thawing cycles can denature an antibody (how?) **causing it to form aggregates that reduce the antibody's binding capacity.**

Storing at -20°C should be adequate for most antibodies; there is no appreciable advantage to storing at -80°C . Antibody vials should be placed in an area of the freezer that has minimal temperature fluctuations, for instance towards the back rather than on a door shelf.

Some researchers add the **cryoprotectant glycerol** to a final concentration of 50% (**Why?**) **to prevent freeze/thaw damage; glycerol will lower the freezing point to below -20°C .** Diluting antibodies to working concentration and storing at 4°C for more than a day should be avoided (**Why?**) Because **proteins in general are less susceptible to degradation when stored at higher concentrations, ideally 1 mg/ml or higher.**