

# Antibody storage guide

Storage temperature, contamination,  
freeze/thaw damage and stability

Please always check datasheets for specific storage recommendations. We are not able to guarantee antibodies that have not been stored correctly. With proper storage and handling, most antibodies should retain activity for months, if not years.

## Storage temperatures

For many of our antibodies, freezing at -20°C or -80°C in small aliquots is the optimal storage condition. Aliquoting minimizes damage due to freezing and thawing, as well as contamination introduced by pipetting from a single vial multiple times. Aliquots should be frozen and thawed once, with any remainder kept at 4°C.

Upon receiving the antibody, centrifuge at 10,000 x g for 20 seconds to pull down solution that is trapped in the threads of the vial, and transfer aliquots into low-protein-binding microcentrifuge tubes. The size of the aliquots will depend on how much you typically use 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.

In most cases storage at 4°C upon receipt of the antibody is acceptable for one to two weeks. It is important to follow the recommendations on the datasheet.

### Exceptions

Enzyme-conjugated antibodies should not be frozen at all and should instead be kept at 4°C. Freezing and thawing will reduce enzymatic activity in addition to affecting the antibody binding capacity.

Conjugated antibodies – whether conjugated to fluorochromes, enzymes, or biotin – should be stored in dark vials or wrapped in foil. Exposure to light will compromise the activity of conjugates. Fluorescent conjugates in particular are susceptible to photo-bleaching and should be protected from light during all phases of an experiment.

Antibodies of the IgG3 isotype are unique in their tendency to form aggregates upon thawing and should always be stored at 4°C.

Ascites fluid may contain proteases, and should be frozen as soon as possible after receipt.

## Contamination

To prevent microbial contamination, sodium azide can be added to an antibody preparation to a final concentration of 0.02% (w/v). Many of our antibodies already contain this preservative at concentrations ranging from 0.02 to 0.05%. This will be indicated on the datasheets in the storage buffer section.

### When not to use sodium azide

If staining or treating live cells with antibodies, or 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 as well: it blocks the cytochrome electron transport system.

Sodium azide will interfere with any conjugation that involves an amine group, and should be removed before proceeding with the conjugation. After conjugation, antibodies can be stored in sodium azide. An acceptable alternative is 0.01% thimerosal (merthiolate), which does not have a primary amine.

Sodium azide can be removed from antibody solutions by dialysis or gel filtration. The molecular weight of IgG is 150,000 daltons (IgM is ~600,000); the molecular weight of sodium azide is 65 daltons. A micro-dialysis unit with a cut off at 14,000 daltons will retain the antibody as the azide diffuses out.

In a beaker on a magnetic stirrer kept at 4°C, use at least a liter of cold PBS per mL of antibody and stir the dialysis unit for 6 hrs. Change the PBS twice, stirring at least 6 h for each change. If possible, all materials should be sterilized and the resulting preparation should be handled aseptically.

## Freeze/thaw damage

Repeated freeze/thaw cycles can denature an antibody, causing it to form aggregates that reduce its binding capacity.

Storing at -20°C should be adequate for most antibodies; there is no appreciable advantage to storing at -80°C. The freezer must not be of the frost-free variety. These cycle between freezing and thawing (to reduce frost-build-up), which is exactly what should be avoided. For the same reason, 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% to prevent freeze/thaw damage; glycerol will lower the freezing point to below  $-20^{\circ}\text{C}$ . While this may be acceptable for many antibodies, only a small percentage of the antibodies we offer have been tested for stability in this storage condition and our guarantee only applies to antibodies stored as recommended on the datasheet. Storing solutions containing glycerol at  $-80^{\circ}\text{C}$  is not advised since this is below the freezing point of glycerol. Please be aware that glycerol can be contaminated with bacteria. If adding glycerol or any cryoprotectant, care should be taken to obtain a sterile preparation.

## Protein concentration and stability

Diluting antibodies to working concentration and storing at  $4^{\circ}\text{C}$  for more than a day should be avoided. Proteins in general are less susceptible to degradation when stored at higher concentrations, ideally 1 mg/mL or higher. This is the rationale for including proteins such as BSA to the antibody solution as stabilizers. The added protein also serves to minimize loss of antibody due to binding to the vessel wall. Do not add stabilizing protein to antibodies that you intend to conjugate, because they will compete with the antibody and reduce the efficiency of the conjugation.