

Antibody Storage and Handling – GaoLab

1. General principles

- a. **Antibodies are precious resources** and must be **conserved**—i.e., use as low concentration as possible. **DO NOT WASTE.**
- b. Antibodies are proteins and should be **kept cold** (refrigerated, on ice, or frozen) when not in use.
- c. The **more dilute** the antibody is, the **less stable**. Therefore, it is good idea to store antibodies in concentrated form without dilution.
- d. Companies that supply antibodies generally also supply instructions on storage and handling. **Read and follow the instructions but the recommended concentration is always higher than it should be.** So optimal concentration should be tested for every new antibody (please refer to published literature).
- e. Antibodies work best when freshly diluted. Do not dilute your antibodies to make working solutions for immunostaining until you're ready to start the incubations.
- f. **Repeated freezing and thawing kills antibodies.** Once you have thawed an antibody solution, store it at 4°C for repeated use (unless you are aliquoting a newly arrived antibody; see next section).

2. What to do with newly arrived antibodies: ALIQUOT!

- a. If the antibody arrives frozen, thaw it and place it on ice. If it is in liquid, immediately place it on ice.
- b. **Gently vortex** the antibody solution and **spin briefly** (at 5,000-10,000 x g for 10 seconds to pull down solution) in microcentrifuge to collect all liquid at bottom of tube.
- c. Check the total volume of the antibody solution and determine how many aliquots you can make. Typically, aliquots of 5-10 µl (or 20 µl) are appropriate (larger aliquots for antibodies that are used with relatively low dilution, e.g. 1:100; and smaller aliquots for antibodies that will be highly diluted, e.g. 1:5000).
- d. **Label** tubes with the antibody name, dilution, volume, and date aliquoted; e.g., anti-NR1, undiluted (1:2), 10 µl, 9/28/2013.
- e. Aliquot the antibody into the **BOTTOM** of tubes. Put the tubes in a box in the freezer, and make sure the box is appropriately labeled (e.g., GaoLab polyclonal antibodies, cat. #, company.).
- f. **Enter the antibody in computer database. Put any accompanying literature or instructions in our antibody notebook or keep a copy for your own record.**
- g. If you thaw an antibody, write the thaw date on the tube; e.g., thaw 9/28/13.

3. Preparing antibodies for immunohistochemistry

- a. **Use thawed antibody stocks first**, but only if less than 3-6 months old.
- b. If you need more antibodies, thaw a new aliquot, **write the thaw date on the tube**, and thereafter store in the refrig. (i.e., do not re-freeze).

4. Preventing contamination with sodium azide (modified from Abcam):

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%. This will be indicated on the datasheets in the section titled "Storage buffer". If not, you can add 0.02% sodium azide (final concentration) into primary antibody if you re-collect and repeatedly use it and store it at 4°C refrigerators.

When NOT to use sodium azide:

- If staining or treating **live cells** with antibodies (e.g. cell culture), or if using antibodies for *in vivo* studies (e.g., intracellular recording), 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 but 0.01% thimerosal (merthiolate), which does not have a primary amine, is an acceptable alternative.

5. Exceptions and other special conditions:

- **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.
- **IgG3 isotype antibodies** are unique in their tendency to form aggregates upon thawing and should always be stored at 4°C.

Again, it is important to follow the recommendations on the datasheet.