

Sodium Azide Removal Protocol

Sodium azide is a preservative used for inhibiting the growth of contaminants such as bacteria or fungi in antibody solutions. However, its presence in antibody solutions can affect the use of the antibody in cell culture assays as it is toxic to cells. It can also interfere with antibody conjugation and also inhibits the activity of the enzyme horseradish peroxidase.

The following three procedures can be used to remove azide:

Dialysis

A dialysis unit can be used to remove azide from samples of 0.1 ml to 70 ml in volume. This is a semi-permeable membrane available in a wide range of size dimensions and pore sizes. Using a membrane with a pore size cut-off at 10,000 to 30,000 Daltons will allow the azide to pass through the membrane but will retain the antibody and other proteins in the solution. Note: The molecular weight of IgG is 150,000 Daltons (IgM is ~ 600,000). The molecular weight of sodium azide is 65 Daltons.

Procedure

- A, Assemble the dialysis unit, as recommended by the manufacturer. Pre-condition the unit for a minimum of 1-2 minutes in the dialysis buffer to allow the membrane to hydrate
- B, Transfer the antibody solution into the dialysis unit
- C, Place the dialysis unit into a suitably sized beaker containing at least 1 liter of buffer against which the antibody is to be dialyzed. Place the beaker on a magnetic stirrer and dialyze for a minimum of 1 hr at 4°C
- D, Change the buffer and dialyze again for at least 1 hr. Repeat until the desired number of buffer changes has been achieved. Ensure that the buffer is changed at least 3 times.

If possible, all materials should be sterilized and the resulting preparation handled aseptically. Cold conditions are recommended as the antibody is no longer protected by preservative.

Desaltin

This procedure is suitable for smaller volumes of 1–3 ml. Desalting resins have size exclusion properties and consist of small particles with a range of pore sizes. Size exclusion is a method used to separate molecules in solution by their molecular weight. Particles of varying molecular weight will elute through a size exclusion matrix at different rates. For example, large molecules cannot enter the pores of the matrix and therefore are eluted first, whereas smaller molecules will penetrate the pores within the beads and elute later.

Procedure

- A, If commercially available purification columns are being used, please refer to the manufacturer's instructions for use
- B, Remove the cap from the spin column and centrifuge at 1000 g for 2 minutes to remove the storage solution
- C, Put the column in a collecting tube
- D, Fill the column with equilibration buffer as advised by the manufacturer and centrifuge at 1000 g for 2 minutes
- E, Repeat 3 times and discard the collected flow-through
- F, Add 1-3 ml of antibody sample slowly to the middle of the packed bed and centrifuge at 1000 g for 2 minutes
- G, Collect and recover the eluate (antibody) located in the collection tube

Antibody purification kit

Kits are commercially available for purification of antibodies which can also be used to remove azide. These kits can be used to purify the antibody from a solution containing BSA, glycine, Tris or sodium azide. It can also be used to purify antibodies from crude samples such as ascites fluid or immune serum. The method involves capture of the antibody on a Protein A resin and removal of unwanted substances by a simple wash procedure, which can be performed using a standard microtube. The purified product is then eluted and neutralized.

For research purposes only ! Not for therapeutic or diagnostic purposes in humans or animals !