Azide Removal by Dialysis Protocol

Rockland and other manufacturers often supply antibodies in buffers that contain sodium azide. While preservatives like azide are generally warranted—in that, they extend the shelf life of an antibody and protect the antibody against contaminating growth—they can prevent the immediate use of the antibody in a limited number of specialized assays. For example, sodium azide can interfere with assays involving cell culture. Sodium azide will also inhibit the enzyme horseradish peroxidase if the antibody is not diluted before use. Rockland never adds sodium azide to peroxidase conjugated antibodies. Fortunately, sodium azide can be easily removed from the buffer by dialysis. When doing this, care should be taken to avoid diluting the antibody solution too much without adding protective protein. Dialysis can be performed either with dialysis tubing for large amounts or via centrifugation for small samples (AMICON and other companies make several very convenient devices for this purpose).

I. Reagents Required

Reagent	Preparation
Antibody solution to be dialyzed	N/A
Dialysis membrane (12,000–14,000 Dalton Molecular Weight Cutoff (MWCO)) SPECTRUM or equivalent	N/A
Dialysis Buffer. We suggest using our 10X PBS pH 7.2 (MB-008) (0.2 M Potassium Phosphate, 1.5 M Sodium Chloride). And diluting appropriated with deionized water.	N/A
Large beaker	N/A
Magnetic stir plate and stir bar	N/A
Cold room or refrigerated cabinet	N/A

II. Procedure

1. For IgG antibodies use 12,000–14,000 Dalton MWCO dialysis tubing. This will allow the azide to pass through the membrane; however, proteins (including the antibody and any other proteins present in the buffer) will not pass through.
2. Transfer the antibody solution to pre-wet dialysis tubing of the appropriate diameter and length to accommodate the volume. Knot one end of the tubing with 2–3 knots or use a closure.
   Note: We suggest pre-filling the tubing with buffer to test for any leaks, draining out the buffer and filling the tubing with antibody solution, then knotting the other end.
3. Place the dialysis tubing into a suitably sized beaker containing the buffer against which the antibody is to be dialyzed.
   Note: A few mL of antibody is dialyzed against a few liters of azide free buffer such as PBS.
4. Dialyze at 4°C with stirring (use a magnetic stirrer) for at least 6 hours per exchange. Change the buffer 3 times at convenient intervals. Each change of buffer will dilute the azide level further.
   Note: Cold temperatures are required because the antibody is no longer protected by preservative! Azide can also be removed using gel filtration column-based methods. (Sephadex® G-25 column system will effectively remove the azide.)

III. Notes

Most methods will result in some loss of protein during transfer by adherence to surfaces.