IgM antibody labeling protocol

1. Prepare your antibody in reaction buffer: 0.1 M sodium phosphate, pH 7.2 to 7.5, with 150 mM NaCl for improved antibody solubility (PBS is ideal). The amount of antibody needed for a labeling reaction is 50 µg to 2.5 mg, dissolved in 100–500 µL.

   Note: Antibodies supplied in PBS, pH 7.4, with 0.1% (or less) sodium azide (NaN₃) can be used directly in the labeling reaction. Buffers containing primary amines (e.g., Tris or glycine) will strongly interfere because they react with the NHS-ester moiety. If your IgM molecule is suspended in such a buffer, it must first be dialyzed against reaction buffer.

2. Transfer 100–500 µL of antibody solution to the vial containing the dye. Mix well and incubate at room temperature for 1 hour. Because the Alexa Fluor® amine-reactive dyes are relatively hydrophilic (especially the SDP ester), it is not necessary to dissolve the dye in organic solvent.

   Alternatively, 1 mg of reactive dye may be dissolved in 10–100 µL of anhydrous DMSO, and the antibody solution added to this dye solution. The Alexa Fluor® dye will hydrolyze more slowly in DMSO, and therefore this may be a preferable solvent if the dye will not be used immediately.

3. Remove unconjugated dye by passing the reaction solution over a short gel filtration column (e.g., BioSpin #732-6008, Bio-Rad). Alternatively, dialyze at 2–6 °C for 12–24 hours with four buffer changes.

4. Store the labeled antibody protected from light at 2–6 °C. We generally do not recommend frozen storage of antibody conjugates; if you wish to store your labeled antibody at −20 °C, we suggest adding 50% (v/v) glycerol to single-use aliquots.

5. For storage at 2–6 °C for longer than a few weeks, we recommend adding sodium azide to a final concentration of 0.02–0.05% (w/v) [0.3–0.75 mM] to inhibit microbial growth.