Reduction of IgG and Biotinylation with Iodoacetyl Biotin Reagent

The following method uses 2-mercaptoethylamine•HCl (2-MEA) as a selective and mild disulfide-cleaving reagent for reducing whole IgG in preparation for biotinylation. The protocol can be modified for other proteins, peptides and other molecules. The protein concentration during the mild reduction is not as critical as the absolute concentration of 2-MEA, which is 50 mM; 1-10 mg IgG/mL can be effectively reduced at this 2-MEA concentration. Generally, a 3- to 5-fold molar excess of iodoacetyl biotin reagent to sulfhydryl groups is sufficient to obtain efficient modification. Specific applications will require optimization of reducing or sulfhydryl addition steps and amount of biotinylation reagent.

Materials Required

1. Sample Preparation Buffer: 0.1 M sodium phosphate, 5 mM EDTA, pH 6.0.
2. 1 mL of 4 mg/mL (27 μM) IgG in Sample Preparation Buffer.
3. 2-Mercaptoethylamine•HCl (2-MEA).
4. Reaction Buffer: 50 mM Tris•HCl, 5 mM EDTA, pH 8.0-8.3.
5. Desalting column.

A. Prepare Reduced IgG

1. Add 1 mL of the IgG solution to the vial containing the 6 mg 2-MEA (results in 50 mM 2-MEA).
2. Mix and incubate the solution for 90 minutes at 37 ℃.
3. Allow the solution to cool to room temperature. Remove the excess 2-MEA from the reduced IgG using a desalting column equilibrated with Reaction Buffer.

B. Biotinylate Reduced IgG With Iodoacetyl Biotin Reagent

1. Immediately before use, prepare 4 mM solution of Iodoacetyl Biotin Reagent.
2. Add 50 μL of the Iodoacetyl Biotin solution per milliliter of the reduced IgG. (This results in 200 μM Iodoacetyl Biotin per 50 μM reduced hinge-region sulfhydryl groups, corresponding to a 4-fold excess of Iodoacetyl Biotin Reagent.).
3. Mix and incubate reaction in the dark for 90 minutes at room temperature.
   Note: Performing the reaction in the dark limits conversion of liberated iodide ions to molecular iodine, which can react with tyrosine residues.
4. Remove non-reacted Biotin Reagent by applying mixture to a desalting column that has been equilibrated with Reaction Buffer. Collect 0.5 mL fractions and monitor for the presence of protein by measuring the absorbance at 280 nm. The first absorption peak emerging from the column corresponds to fractions containing the biotinylated IgG. Alternatively, the non-reacted Biotin Reagent may be removed by dialysis.

Notice

Iodoacetyl-LC-Biotin is moisture-sensitive. If the vial of reagent has been stored cold, fully equilibrate vial to room temperature before opening to avoid moisture condensation inside the container. Do not prepare stock solutions for storage, and dissolve the biotin reagent immediately before use. Perform reactions in buffers that are free of thiols.