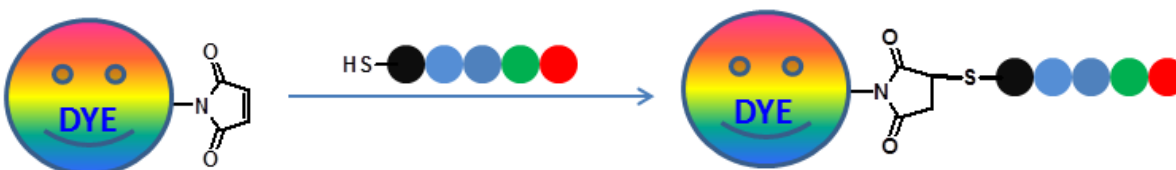


Cal-520™ Maleimide

Introduction

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Cal-520 has been proven to be the best green fluorescent calcium indicator. Thiol-reactive Cal-520 maleimide can be coupled to an IgG or other carrier molecules to prepare a calcium-sensitive bioconjugate for monitoring calcium change spatially for a specific target. Because free thiol (SH) groups are not present as abundantly as amino groups in most proteins, thiol-reactive reagents provide a means of selectively modifying a protein at a defined site. Cal-520™ maleimide readily reacts with thiol moieties of small molecules or biopolymers to form thioether conjugates even under neutral conditions. The thioether bond formed is quite stable.



Storage and Handling

Upon receipt, Cal-520™ maleimide should be stored at $<-15^{\circ}\text{C}$, and kept from light and moisture. The reconstituted DMSO stock solution of Cal-520™ maleimide can be stored at $<-15^{\circ}\text{C}$ for up to 4 weeks. The desired protein conjugate should be stored at >0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). The protein conjugate solutions could be stored at 4°C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer storage, the protein conjugates could be lyophilized or divided into single-used aliquots and stored at $\leq -60^{\circ}\text{C}$, and protected from light.

Sample Protocol for Labeling Proteins

Note: This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with Cal-520™ maleimide. You might need further optimization for your particular proteins.

1. Prepare protein stock solution (Solution A):

1.1. (Optional) if your protein does not contain a free cysteine, you must treat your protein with DTT or TCEP to generate a thiol group. DTT or TCEP are used for converting a disulfide bond to two free thiol groups. If DTT is used you must remove free DTT by dialysis or gel filtration before conjugating a dye maleimide to your protein. Following is a sample protocol for generating a free thiol group:

- Prepare a fresh solution of 1 M DTT (15.4 mg/100 μl) in distilled water.
- Make IgG solution in 20 mM DTT: add 20 μl of DTT stock per ml of IgG solution while mixing. Let stand at room temp for 30 minutes without additional mixing (to minimize reoxidation of cysteines to cystines).
- Pass the reduced IgG over a filtration column pre-equilibrated with "Exchange Buffer". Collect 0.25 ml fractions off the column.
- Determine the protein concentrations and pool the fractions with the majority of the IgG. This can be done either spectrophotometrically or colorimetrically.
- Carry out the conjugation as soon as possible after this step (see below).

Note 1: IgG solutions should be >4 mg/ml for the best results. The antibody should be concentrated if less than 2 mg/ml. Include an extra 10% for losses on the buffer exchange column.

Note 2: The reduction can be carried out in almost any buffers from pH 7-7.5, e.g., MES, phosphate or TRIS buffers.

Note 3: Steps c and d can be replaced by dialysis.

1.2. Dissolve your protein in a reaction buffer (e.g., 100 mM MES buffer with pH ~6.0) to give the protein labeling stock solution (protein concentration >1 mg/ml if possible).

***Note 1:** The pH of the protein solution should be 6.5 ± 0.5 .*

***Note 2:** Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or other proteins will not be labeled well.*

***Note 3:** The conjugation efficiency is significantly reduced if the protein concentration is less than 1 mg/mL. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.*

2. Run conjugation reaction:

2.1 Calculate the amount of protein solution to be used. We recommend the **molar** ratio of Cal-520/protein to be 5-10.

2.2 Add proper amount of protein labeling solution (from Step 2.1) into the vial of Cal-520™ maleimide (0.1 μmol).

2.3 Mix well by pipetting or vortex. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

3. Purify the conjugation

The unreacted Cal-520™ maleimide can be readily removed by a spin column.