N-Sp-DMAE, Maleimide

Catalog number: 26012
Unit size: 1 mg

Component	Storage	Amount (Cat No. 26012)
N-Sp-DMAE, Maleimide	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

N-Sp-DMAE, Maleimide is a thiol-reactive acridinium-based chemiluminescent labeling reagent that enables covalent attachment to cysteine residues in proteins and other sulfhydryl-containing biomolecules. Upon exposure to alkaline hydrogen peroxide, the acridinium moiety produces a rapid flash-type chemiluminescent signal, making it suitable for sensitive detection in immunoassays and related analytical workflows. This reagent is particularly useful for labeling reduced antibodies and other thiol-containing biomolecules. During hydrogen peroxide activation, the acridinium label is released from the carrier molecule as part of the chemiluminescent reaction. For applications requiring the acridinium label to remain attached after signal generation, alternative acridinium ester chemistries may be considered.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

N-Sp-DMAE, Maleimide stock solution (Solution B)

Add anhydrous DMSO into the vial of N-Sp-DMAE Maleimide to make a 10 mM stock solution. Mix well by pipetting or vortex.

Note: Prepare this stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the stock solution may reduce the activity. Solution B can be stored in freezer for upto 4 weeks when kept away from light and moisture. Avoid freeze-thaw cycles.

Protein stock solution (Solution A)

Mix 100 µL of a reaction buffer (e.g., 100 mM MES buffer with pH ~6.0) with 900 µL of the target protein solution (e.g. antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.

Note: The pH of the protein solution (Solution A) should be 6.5 ± 0.5.

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

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

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 maleimide probe to your protein. Following is a sample protocol for generating a free thiol group:

1. Prepare a fresh solution of 1 M DTT (15.4 mg/100 µL) in distilled water.
2. 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).
3. Pass the reduced IgG over a filtration column pre-equilibrated with "Exchange Buffer". Collect 0.25 mL fractions off the column.
4. Determine the protein concentrations and pool the fractions with the majority of the IgG. This can be done either spectrophotometrically or colorimetrically.
5. Carry out the conjugation as soon as possible after this step (see Sample Experiment Protocol).

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

   Note: Steps 3 and 4 can be replaced by dialysis.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with N-Sp-DMAE maleimide. You might need further optimization for your particular proteins.

Note: Each protein requires distinct label/protein ratio, which also depends on the properties of labels. Over labeling of a protein could detrimentally affects its binding affinity while the protein conjugates of low label/protein ratio gives reduced sensitivity.

Run conjugation reaction

1. Use 10:1 molar ratio of Solution B (label)/Solution A (protein) as the starting point: Add 5 µL of the label stock solution (Solution B, assuming the stock solution is 10 mM) into the vial of the protein solution (95 µL of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/mL and the molecular weight of the protein is ~200KD.

   Note: We recommend to use 10:1 molar ratio of Solution B (label)/Solution A (protein). If it is too less or too high, determine the optimal label/protein ratio at 5:1, 15:1 and 20:1 respectively.

2. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the conjugation

The following protocol is an example of conjugate purification by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture instruction.
2. Load the reaction mixture (From "Run conjugation reaction") to the top of the Sephadex G-25 column.
3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top

resin surface.

4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired label-protein conjugate.

   Note: For immediate use, the conjugated protein needs be diluted with staining buffer, and aliquoted for multiple uses.

   Note: For longer term storage, the conjugate solution need be concentrated or freeze dried.

EXAMPLE DATA ANALYSIS AND FIGURES

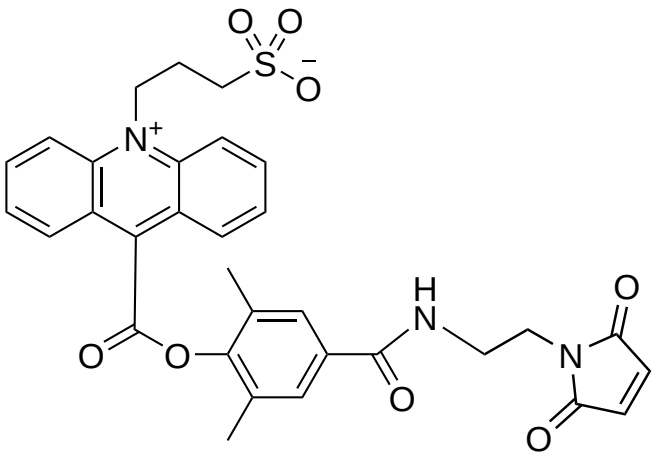

Figure 1. Chemical structure for N-Sp-DMAE, Maleimide.

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