Digoxigenin NHS ester

Catalog number: 3500, 3501
Unit size: 1 mg, 5 mg

Component	Storage	Amount (Cat No. 3500)	Amount (Cat No. 3501)
Digoxigenin NHS ester	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)	5 mg

OVERVIEW

Digoxigenin NHS Ester is an activated ester reagent designed for covalent attachment of digoxigenin (DIG) to amino-containing biomolecules. Digoxigenin is a highly antigenic hapten used in biological detection workflows, similar to other small-molecule tags such as 2,4-dinitrophenol (DNP) and biotin. The NHS ester group reacts with primary amines under mild conditions, enabling DIG labeling of proteins, peptides, amino-modified oligonucleotides, and related biomolecules. DIG-labeled biomolecules are commonly detected using anti-digoxigenin antibodies with fluorescent, enzyme, or other reporter labels. This labeling strategy is useful for preparing tagged probes or conjugates for immunoassays, nucleic acid detection, hybridization-based assays, and other biological detection applications where anti-DIG antibody recognition provides signal generation.

AT A GLANCE

PREPARATION OF STOCK SOLUTIONS

Protein Solution Preparation

1. Prepare a protein stock solution in PBS.Prepare a protein stock solution in PBS.
2. Add 10% v/v 1.0 M sodium bicarbonate solution or 1.0 M phosphate buffer with (pH ~8.5) to adjust pH to ~8.5.

Notes:

1. The pH of the protein solution should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using 1.0 M sodium bicarbonate solution or 1.0 M pH 9.0 phosphate buffer.

2. The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4. If the protein is already dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

3. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.

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

Reactive Digoxigenin Solution Preparation

Immediately before use, dissolve the amine-reactive compound in high-quality, anhydrous dimethylsulfoxide (DMSO) at a final concentration of 10 mg/mL. Once reconstituted, use the reactive solution immediately. Extended storage of the stock solution may reduce the activity. The stock solution can be stored in the freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

Calculate the Amount of the Reactive Digoxigenin Needed:

Based on the protein concentration, select a desired labeling ratio, and calculate the amount of dye needed using this equation:

Amount of label (mg) = (Amount of protein (mg) / MW of the protein) x (Labeling Ratio) x (MW of the label)

Note: We recommend to start with 10:1 label to protein molar ratio. If it is too less or too high, determine the optimal label/protein ratio at 5:1, 15:1, and 20:1, respectively.

Run Conjugation Reaction:

While stirring the protein solution, slowly add the reactive digoxigenin solution. Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the Conjugate:

1. Select a desalting column based on the volume of reaction.

   Note: For 50-100uL reaction mixture, the recommended column is https://www.aatbio.com/products/readiuse-bio-gel-p-6-spin-column or similar and for 0.6mL to 1mL reaction mixture, the recommended column is https://www.aatbio.com/products/readiuse-10kd-spin-filter?unit=60502 or similar.

2. Equilibrate with at least three column volume (CV) of with PBS.
3. Separate the conjugate on the desalting column according to the manufacture instruction.
4. Load the reaction mixture to the column (≤ 10% of column volume).
5. Add more PBS (pH 7.2-7.4) or centrifuge to complete the column purification.
6. Combine the fractions that contain the desired dye-protein conjugate.

   Note: Store the protein conjugates under the same conditions as the un-labeled protein. For immediate use, the conjugated protein must be diluted with staining buffer, and aliquoted for multiple uses. For longer-term storage, the conjugated protein solution needs to be concentrated or freeze-dried.

Characterize the Protein Concentration:

Measure absorption with nanodrop and determine the concentration of the protein in mg/mL based on the Ec of the protein.

EXAMPLE: BSA LABELING WITH DIGOXIGENIN NHS ESTER

1. Protein Solution Preparation:

Amount of Protein: 0.5 mg BSA, 5 mg/ml in PBS, 100 µL

MW of BSA: 66500

Labeling Ratio: 10

Label: Digoxigenin NHS ester

MW of Label: 658.79

2. Calculate amount of Digoxigenin NHS ester needed:

The amount of Digoxigenin NHS ester needed:

(0.5 mg /66500) x 10 x 658.79 = 0.050 mg

Volume (µL) needed = 5.0 µL

3. Conjugation Protocol:

Prepare 100 µL BSA in PBS (5 mg/ml), add 10 µL of 1.0M NaHCO3 (pH=8.5), and then add 5 µL of Digoxigenin NHS ester (10 mg/ml in DMSO). Incubate the reaction for 60 min, and then use a P-6 spin column to purify the conjugate to PBS (follow manufacture's instruction). Finally collect 105 µL BSA-Digoxigenin conjugate.

4. Characterize the Protein Concentration:

Measure absorbance of the conjugate with Nanodrop, A280 nm= 2.712

BSA- Digoxigenin conc. (mg/ml) = 2.712/0.667 = 4.1 mg/ml

(Mass Extinction Coefficient of BSA= Ec 0.1% at 280 nm: ~0.667 mL mg^{-1} cm^{-1}).

EXAMPLE DATA ANALYSIS AND FIGURES

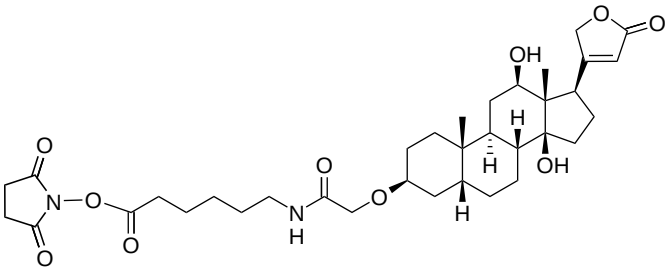

Figure 1. Chemical structure for Digoxigenin NHS ester

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