Lumiwox™ acridinium NHS ester

Catalog number: 26000
Unit size: 1 mg

Component	Storage	Amount (Cat No. 26000)
Lumiwox™ acridinium NHS ester	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

The acridinium NHS ester can be used to label proteins and nucleic acids. The covalently bound acridinium NHS esters generate chemiluminescence in the presence of hydrogen peroxide. Exposure of an acridinium ester label to an alkaline hydrogen peroxide solution triggers a flash of light. Acridinium-labeled proteins and other biological molecules can be used as sensitive detection methods in immunoassays and other biological detections. Compared to the other commercial acridinium NHS esters, the conjugates prepared from Lumiwox™ acridinium NHS ester generate stronger luminescence signals. In addition, the conjugates derived from Lumiwox™ acridinium NHS ester still maintain their acridinium tags attached to their target molecules upon hydrogen peroxide-induced chemiluminescence reaction while the conjugates derived from the other commercial acridinium NHS esters lose their acridinium tags upon hydrogen peroxide-induced chemiluminescence reaction. This critical feature enables Lumiwox™ acridinium probe useful for biological detections on various surfaces, e.g., microarrays etc.

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

Protein stock solution (Solution A)

1. Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g., antibody, protein concentration >2 mg/mL if possible) to give a 1 mL protein labeling stock solution.

   Note: The pH of the protein solution (Solution A) 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 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.

   Note: The protein should be dissolved in 1X phosphate-buffered saline (PBS), pH 7.2-7.4. If the protein is 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.

   Note: 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.

   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.

Lumiwox™ acridinium NHS ester stock solution (Solution B)

1. Add anhydrous DMSO into the vial of Lumiwox™ acridinium NHS ester to make a 10 mM stock solution. Mix well by pipetting or vortex.

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

SAMPLE EXPERIMENTAL PROTOCOL

This protocol describes how to conjugate goat anti-mouse IgG with Lumiwox™ acridinium NHS ester. Further optimization may be necessary for your specific protein.

Note: Each protein requires a distinct dye/protein ratio, which also depends on the properties of dyes. Over-labeling of a protein could detrimentally affect its binding affinity, while the protein conjugates of low dye/protein ratio give reduced sensitivity.

Run conjugation reaction

1. Use a 10:1 molar ratio of Solution B (dye)/Solution A (protein) as the starting point: Add 5 µL of the dye stock solution (Solution B, assuming the dye 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 using a 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too low or too high, determine the optimal dye/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 dye-protein 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 dye-protein conjugate.

   Note: For immediate use, the dye-protein conjugate needs to be

diluted with staining buffer, and aliquoted for multiple uses.

Note: For longer-term storage, the dye-protein conjugate solution needs to be concentrated or freeze-dried.

EXAMPLE DATA ANALYSIS AND FIGURES

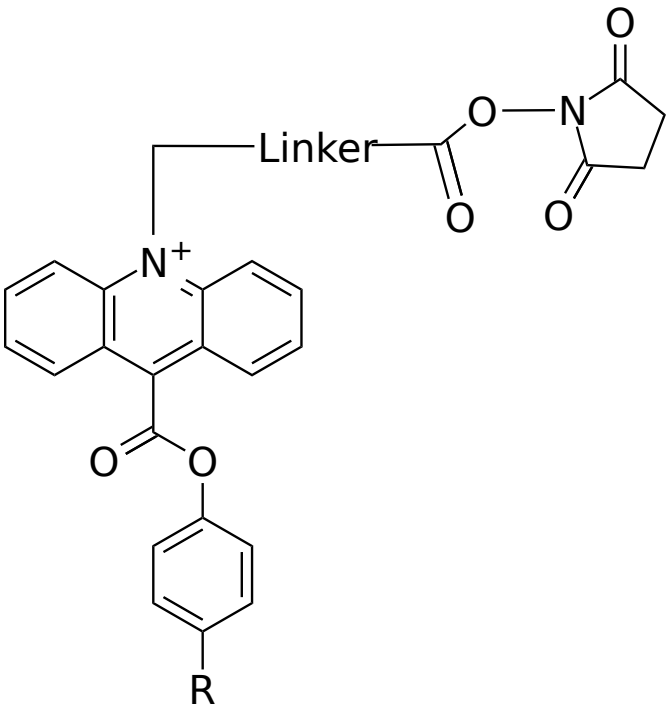

Figure 1. Chemical structure for Lumiwox™ acridinium NHS ester.

DISCLAIMER

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