ReadiLeave™ Reversible Biotin Maleimide

Catalog number: 3402
Unit size: 1 mg

Component	Storage	Amount (Cat No. 3402)
ReadiLeave™ Reversible Biotin Maleimide	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

ReadiLeave™ Reversible (RLR) Biotin is a newly developed biotin derivative that has significantly reduced affinity to avidin (including streptavidin) to make the binding of RLR biotin and streptavidin readily reversible when needed. It is complimentary to the regular biotin and has a moderate affinity to streptavidin to ensure a tight binding but not too tight to be reversed in contrast with the regular non-reversible biotin. ReadiLeave™ Reversible Biotin Maleimide is an excellent building block to develop reversible biotin probes and products for biological detections and purification. It readily reacts with a thiol-containing molecule under neutral to slightly acidic conditions with high yield. The affinity between streptavidin and biotin might be the strongest non-covalent interactions known in biological interactions. Streptavidin, a homotetrameric protein, exhibits an extraordinarily high affinity for biotin. Each streptavidin monomer can bind one biotin molecule, allowing a streptavidin protein to maximally bind four biotins. The streptavidin-biotin interaction is highly specific and remains robust under a wide range of conditions. Biotin can readily be attached to proteins, nucleic acids, or even nanoparticles. Once formed, the bond between biotin and streptavidin is unaffected by extremes of pH, temperature, organic solvents, and other denaturing agents. This powerful interaction has been exploited for various applications such as ELISA, Western blotting, Northern blotting, Southern blotting, immunohistochemistry (IHC), cell surface labeling, Fluorescence-Activated Cell Sorting (FACS), and electrophoretic Mobility Shift Assays (EMSA) 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

Prepare Protein Solution

1. Prepare a 900 uL protein solution in 1X phosphate-buffered saline (PBS), pH 7.2-7.4.

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

   Note: Protein solution should be free of stabilizers like bovine serum albumin (BSA) or gelatin.

   Note: The presence of sodium azide or thimerosal might also interfere with the conjugation reaction.

   Note: The protein concentration range of 2-10 mg/mL is recommended for optimal labeling efficiency.

Optional: Disulfide Reduction

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 converts disulfide bonds to two free thiol groups. If you use DTT, you must remove free DTT by dialysis or gel filtration before conjugating the ReadiLeave™ Reversible Biotin Maleimide to your protein. The 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. To make an IgG solution in 20 mM DTT, add 20 µL of DTT solution per 1 mL of IgG solution while mixing well. Allow the solution to stand at room temperature for 30 minutes without additional mixing to reduce the oxidation of cysteines to cystines.

3. Pass the reduced IgG through the filtration column that has been pre-equilibrated with "Exchange Buffer." Collect 0.25 mL fractions from 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. It is recommended to carry out the conjugation immediately after this step. Please refer to the Sample Experiment Protocol for more details.

   Note: IgG solutions should be >4 mg/mL for the best results. The protein should be concentrated if less than 2 mg/mL. An additional 10% should be included 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.

Prepare ReadiLeave™ Reversible (RLR) Biotin Maleimide Stock Solution

1. Add anhydrous DMSO into the vial of RLR Biotin maleimide to make a 10 mM (6.85 mg/mL) stock solution. Mix well by pipetting or vortexing.

   Note: Prepare the dye stock solution before starting the conjugation. Use promptly.

   Note: RLR Biotin maleimide stock solution can be stored in the freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

   Note: Extended storage of the dye stock solution may reduce the dye activity.

SAMPLE EXPERIMENTAL PROTOCOL

Run Conjugation Reaction

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with RLR Biotin Maleimide.

1. Use a 10:1 molar ratio of RLR Biotin Maleimide:Protein.

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

Purify the Conjugate

1. Purify the conjugate mixture to 1x PBS buffer (pH=7.2-7.4) with a ReadiUse™ Disposable PD-10 Desalting Column (Cat no. 60504) according to the manufacturer's instruction.

Measure Protein Concentration

1. Protein concentration can be determined from the extinction coefficient by measuring absorbance at 280 nm.

Protocol for Target Protein Pull-down Assays

Section 1: Coupling RLR Biotinylated Protein to a Resin

1. Select a streptavidin-resin suitable for your application.
2. Wash and equilibrate the resin by adding 1xPBS or a suitable wash buffer.
3. Add appropriate amounts of RLR Biotinylated protein and incubate for 30 minutes.
4. Wash the resin to remove unlabeled protein and equilibrate with PBS.

Section 2: Pull-down the Target Protein

1. Add the sample containing the target protein to the resin from Section 1 above.
2. Incubate for 60 minutes.
3. The target protein will be pulled down by RLR Biotinylated protein resin from Section 1.

Section 3: Elution of the Target Protein

1. Centrifuge the resin to remove the supernatant and wash the resin by adding 1xPBS buffer (pH=7.2~7.4) or a suitable wash buffer.
2. Repeat washing as needed.
3. Add elution buffer (4 mM d-biotin in 20 mM Tris-HCl Buffer (pH=7.5) with 50 mM NaCl) and incubate at 37°C for 10 minutes or longer. Repeat three times or as needed.
4. Pool all the elution, and the target protein and RLR biotinylated protein complex will be ready for further analysis.

EXAMPLE DATA ANALYSIS AND FIGURES

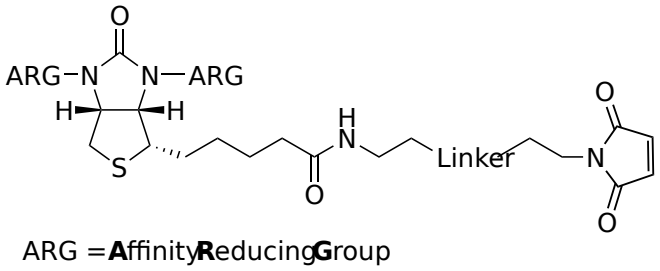

Figure 1. ReadiLeave™ Reversible (RLR) Biotin is a newly developed biotin derivative that has significantly reduced affinity to avidin (including streptavidin) to make the binding of RLR biotin and streptavidin readily reversible when needed. ReadiLeave™ Reversible Biotin Maleimide is an excellent building block to develop reversible biotin probes and products for biological detections and purification.

DISCLAIMER

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