

ReadiLeave™ Reversible Biotin Succinimidyl Ester

 Catalog number: 3400, 3401
 Unit size: 1 mg, 5 mg

Component	Storage	Amount (Cat No. 3400)	Amount (Cat No. 3401)
ReadiLeave™ Reversible Biotin Succinimidyl Ester	Freeze (< -15 °C), Minimize light exposure	1 mg	5 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. ReadiLeave™ Reversible Biotin Succinimidyl Ester is an excellent building block to develop reversible biotin probes and products for biological detections and purification. 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 µL protein solution in 1X phosphate-buffered saline (PBS), pH 7.2-7.4.

Note: If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4.

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.

2. Add 100 µL of a reaction buffer (e.g., 1 M sodium bicarbonate solution or 1 M phosphate buffer with pH ~8.5 to 9.0) to the target protein solution to adjust pH to 8.5 ± 0.5.

Prepare ReadiLeave™ Reversible (RLR) Biotin Succinimidyl Ester Stock Solution

1. Add anhydrous DMSO into the vial of RLR Biotin SE to make a 10 mM (6.85mg/ml) stock solution.

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

Note: RLR Biotin SE 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 SE.

1. Use a 10:1 molar ratio of RLR Biotin SE: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 the above section.
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

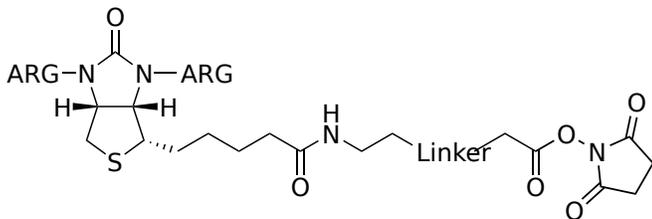


Figure 1. ReadILeave™ Reversible (RLR) Biotin is a newly developed biotin derivative that has significantly reduced affinity to avidin (including streptavidin) to make the binding of RLR biotin and streptavidin readily reversible when needed. It is complimentary to the regular biotin and has a moderate affinity to streptavidin to ensure a tight binding but not too tight to be reversed.

DISCLAIMER

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