

**ReadiLeave™ Reversible Biotin Azide**

Catalog number: 3403

Unit size: 1 mg

Component	Storage	Amount (Cat No. 3403)
ReadiLeave™ Reversible Biotin Azide	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 Azide is an excellent building block to develop reversible biotin probes and products for biological detections and purification using the well-known click reactions (CuAAC). It readily reacts with an alkyne-modified biomolecule under mild conditions. 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*

**Required Stock Solutions for Labeling Alkyne-Modified Biomolecules**

- 200 mM THPTA [tris (3-hydroxypropyl)triazolylmethyl) amine] ligand in water
- 100 mM CuSO<sub>4</sub> in water
- 100 mM sodium ascorbate in water
- 10 mM ReadiLeave™ Reversible (RLR) Biotin Azide in DMSO

**Required Stock Solutions for Labeling Cells, Cell Lysates, or Biological Samples**

- 100 mM THPTA [tris (3-hydroxypropyl)triazolylmethyl) amine] ligand in aqueous buffer or water
- 20 mM CuSO<sub>4</sub> in water
- 300 mM sodium ascorbate in water
- 5 mM ReadiLeave™ Reversible (RLR) Biotin Azide in DMSO

**SAMPLE EXPERIMENTAL PROTOCOL**
**Labeling Oligonucleotides with RLR Biotin Azide**

1. Prepare the required stock solutions for labeling alkyne-modified biomolecules from the 'Preparation of Stock Solutions'

section above.

2. Prepare an alkyne-modified oligo in water as concentrated as possible (e.g., >10 mg/mL).
3. Mix and vortex well CuSO<sub>4</sub> with THPTA in a 1:2 ratio for several minutes before the reaction. This working solution is stable for several weeks when frozen.
4. To the alkyne-modified oligo solution, add an excess of RLR Biotin Azide (2-5 equivalents by molar ratio).
5. Add 5 equivalents of THPTA/CuSO<sub>4</sub> working solution (from Step 1).
6. Add 10-30 equivalents of sodium ascorbate.
7. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
8. Ethanol-precipitate the oligo or purify it using your desired method (e.g., HPLC).

**Labeling Peptides with RLR Biotin Azide**

1. Prepare the required stock solutions for labeling alkyne-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an alkyne-modified peptide in water or DMF as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO<sub>4</sub> with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
4. To the alkyne-modified peptide solution, add an excess of RLR Biotin Azide (5-10 equivalents by molar ratio).
5. Add 5-10 equivalents of THPTA/CuSO<sub>4</sub>.
6. Add 10-20 equivalents of sodium ascorbate.
7. Stir, vortex, or shake the reaction mixture at room temperature for 30-60 minutes.
8. Purify your desired peptide by HPLC.

**Labeling Small Organic Alkyne Molecules with RLR Biotin Azide**

1. Prepare the required stock solutions for labeling alkyne-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an alkyne compound in water or DMF as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO<sub>4</sub> with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
4. To the alkyne solution, add an excess of RLR Biotin Azide (5-10 equivalents by molar ratio).
5. Add 25 equivalents of THPTA/CuSO<sub>4</sub>.
6. Add 50 equivalents of sodium ascorbate.

7. Stir the reaction mixture at room temperature for 30-60 minutes.
8. Purify your desired molecule by chromatography or other methods.

### Labeling Biopolymers with RLR Biotin Azide

1. Prepare the required stock solutions for labeling alkyne-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an alkyne-modified biopolymer in water as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO<sub>4</sub> with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
4. To the alkyne-modified biopolymer solution, add an excess of RLR Biotin Azide (Loading ratio: 5-20 RLR Biotin azide).
5. Add 5 molar equivalents (referenced to RLR Biotin Azide) of THPTA/CuSO<sub>4</sub>.
6. Add 10 equivalents of sodium ascorbate (referenced to RLR Biotin Azide).
7. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
8. Purify your desired molecule by gel filtration or dialysis.

### Labeling Cells, Cell Lysates or Biological Samples with RLR Biotin Azide

1. Prepare the required stock solutions for labeling cells, cell lysates, or biological samples from the 'Preparation of Stock Solutions' section above.
2. For each alkyne-modified cell or cell lysate sample, add the following reagents to a 1.5 mL microfuge tube, then vortex briefly to mix:
  - o 50 µL cell or cell lysate sample
  - o 50 µL PBS buffer
  - o 50 µL of 5 mM RLR Biotin Azide in DMSO or water
3. Add 10 µL of 100 mM THPTA solution, and vortex briefly to mix.
4. Add 10 µL of 20 mM CuSO<sub>4</sub> solution and vortex briefly to mix.
5. Add 10 µL of 300 mM sodium ascorbate solution to initiate a click reaction, and vortex briefly to mix.
6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Cells or cell lysates are now click-labeled and ready for downstream processing and/or analysis.

### 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.

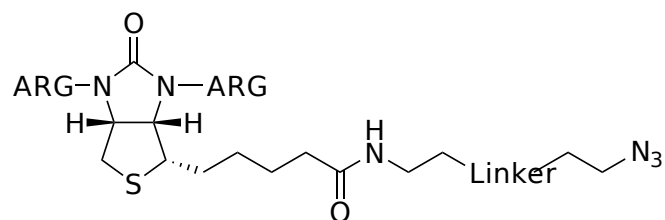
#### Section 2: Pull-down the Target Protein

1. Add a sample containing the target protein to the resin from the Section 1.
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



ARG = **A**ffinity **R**educing **G**roup

**Figure 1.** Readily 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. Readily Reversible Biotin Azide is an excellent building block to develop reversible biotin probes and products for biological detections and purification using the well-known click reactions (CuAAC).

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