ReadiLeave™ Reversible Biotin Alkyne

Catalog number: 3404
Unit size: 1 mg

Component	Storage	Amount (Cat No. 3404)
ReadiLeave™ Reversible Biotin Alkyne	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 Alkyne 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 azido-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.

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 Azide-Modified Biomolecules

1. 200 mM THPTA [tris (3-hydroxypropyltriazolylmethyl) amine)] ligand in water
2. 100 mM CuSO4 in water
3. 100 mM sodium ascorbate in water
4. 10 mM ReadiLeave™ Reversible (RLR) Biotin Alkyne in DMSO

Required Stock Solutions for Labeling Cells, Cell Lysates, or Biologial Samples

1. 100 mM THPTA [tris (3-hydroxypropyltriazolylmethyl) amine)] ligand in aqueous buffer or water
2. 20 mM CuSO4 in water
3. 300 mM sodium ascorbate in water
4. 5 mM ReadiLeave™ Reversible (RLR) Biotin Alkyne in DMSO

SAMPLE EXPERIMENTAL PROTOCOL

Labeling Oligonucleotides with RLR Boitin Alkyne

1. Prepare the required stock solutions for labeling azide-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an azide-modified oligo in water as concentrated as possible (e.g., >10 mg/mL).
3. Mix and vortex well CuSO4 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 azide-modified oligo solution, add an excess of RLR Biotin Alkyne (2-5 equivalents by molar ratio).
5. Add 5 equivalents of THPTA/CuSO4 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 Alkyne

1. Prepare the required stock solutions for labeling azide-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an azide-modified peptide in water or DMF as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO4 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 azide-modified peptide solution, add an excess of RLR Biotin Alkyne (5-10 equivalents by molar ratio).
5. Add 5-10 equivalents of THPTA/CuSO4.
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 Azide Molecules with RLR Boitin Alkyne

1. Prepare the required stock solutions for labeling azide-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an azide compound in water or DMF as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO4 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 azide solution, add an excess of RLR Biotin Alkyne (5-10 equivalents by molar ratio).
5. Add 25 equivalents of THPTA/CuSO4.
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 Boitin Alkyne

1. Prepare the required stock solutions for labeling azide-modified biomolecules from the 'Preparation of Stock Solutions' section above.
2. Prepare an azide-modified biopolymer in water as concentrated as possible (e.g., >10 mg/mL).
3. Incubate CuSO4 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 azide-modified biopolymer solution, add an excess of RLR Biotin Alkyne (Loading ratio: 5-20 RLR Botin Alkyne).
5. Add 5 molar equivalents (referenced to RLR Biotin Alkyne) of THPTA/CuSO4.
6. Add 10 equivalents of sodium ascorbate (referenced to RLR Biotin Alkyne).
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 Boitin Alkyne

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 azide-modified cell or cell lysate sample, add the following reagents to a 1.5 mL microfuge tube, then vortex briefly to mix:
   - 50 µL cell or cell lysate sample
   - 50 µL PBS buffer
   - 50 µL of 5 mM RLR Biotin Alkyne 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 CuSO4 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

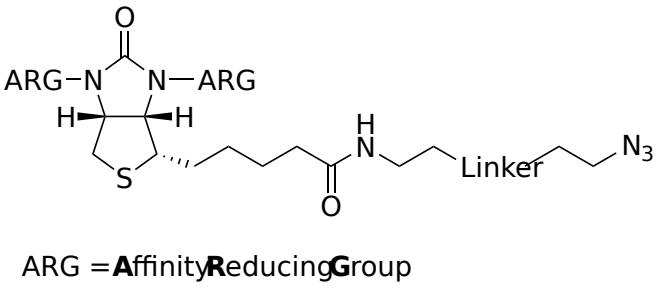

Figure 1. ReadiLeave™ Reversible (RLR) Biotin is a newly developed biotin derivative that has significantly reduced affinity to avidin (including streptavidin) to make the binding of RLR biotin and streptavidin readily reversible when needed. ReadiLeave™ Reversible Biotin Azide is an excellent building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

DISCLAIMER