

Dde Biotin Probes

Dde Biotin Probes are novel hydrazine cleavable biotinylation reagents for labeling alkyne- or azide-containing molecules. Biotinylated protein targets can be selectively captured through conventional affinity biotin - streptavidin purification, and released under mild conditions with a 2% hydrazine solution for subsequent identification by mass spectrometry, ELISA, dot blot, or Western blot applications.

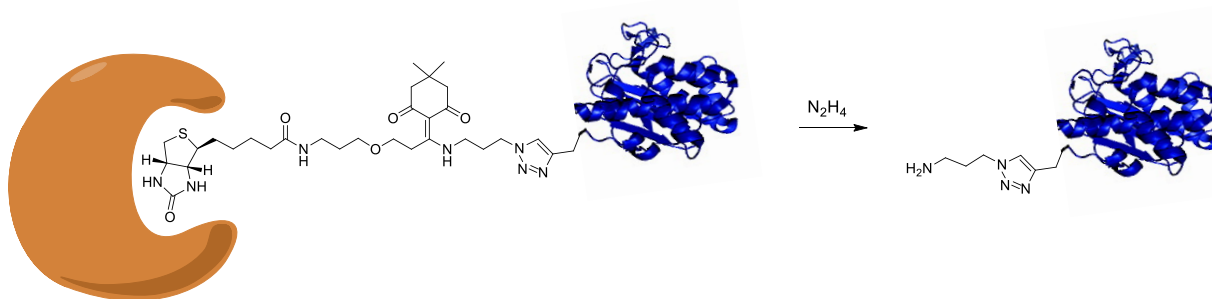


Figure 1. Schematic representation of Dde biotin probe cleavage.

Enrichment Protocol for Dde Biotin Probes

This section contains general protocols using cell lysates. These protocols may be used as a starting point for optimization of your particular click chemistry procedures. The auxiliary reagents and complete kits can be ordered from Click Chemistry Tools.

Procedure for Click Reaction

- Prepare the following click solutions:
 - 100 mM THPTA ligand in aqueous buffer or water
 - 20 mM CuSO₄ in water
 - 300 mM sodium ascorbate in water
 - 2.5 mM alkyne or azide labeling reagent in water or DMSO
- For each azide or alkyne- modified protein lysate sample, add the following to a 1.5 mL microfuge tube, then vortex briefly to mix.
 - 50 µL protein lysate (1-5 mg/mL) in protein extraction buffer
 - 90 µL PBS buffer
 - 20 µL of 2.5 mM corresponding azide (or alkyne) detection reagent in DMSO or water

3. Add 10 μ L of 100 mM THPTA solution, vortex briefly to mix.
4. Add 10 μ L of 20 mM CuSO_4 solution, vortex briefly to mix.
5. Add 10 μ L of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Proteins in lysate are now click labeled and ready for downstream processing and/or analysis.

Procedure for Capture of Biotin Labeled Proteins

The capture protocol provided is optimized for high capacity streptavidin agarose. The typical range for biotin labeled protein is 20-200 μ g of total protein per 100 μ L of settled resin. This can be scaled up or down, depending on the amount of biotin labeled protein to be captured on the resin.

1. Thoroughly mix and vortex the high capacity streptavidin agarose resin to resuspend. Transfer the desired volume of resin to a 1.7 mL microfuge tube. Use a P200 pipette with a cut tip to ensure that the resin is transferred properly.
2. Centrifuge the resin at 500 x g for 2 min to pellet the resin.
3. Carefully remove and discard the storage buffer (contains Kathon as preservative).
4. Resuspend the pellet in an equal volume of PBS pH 7.0 (50% slurry in PBS). Vortex to mix thoroughly. Centrifuge at 500 x g for 2 min. Carefully remove and discard the wash buffer. Repeat this wash step 2 additional times.
5. After equilibration into PBS pH 7.0, add required volume of biotin labeled proteins (see above). The volume ratio of settled resin:labeled protein should not exceed 2:1 (for example, if using 100 μ L of settled resin, do not use less than 50 μ L of protein solution). After addition of biotin labeled proteins, vortex briefly and incubate for 30-120 min at room temperature with agitation.
6. After incubation, centrifuge at 500 x g for 2 min. Retain the supernatant (flow through) for analysis by UV/VIS and/or SDS-PAGE.
7. Wash the resin by the addition of PBS pH 7.0 (use a volume equal to or greater than the volume of resin). Vortex briefly to resuspend the pellet. Centrifuge at 500 x g for 2 min. Carefully remove and discard the supernatant. Repeat this wash step 3-6 times.
8. **Note:** Do not use buffers with pH>7.8 or those that contain SDS as this can cause cleavage.
9. The resin now contains bound biotin labeled protein and is ready for cleavage/elution.

Procedure for Cleavage

1. Resuspend the washed resin in elution buffer (100 mM sodium phosphate + 2%(v/v) hydrazine). **NOTE:** hydrazine solutions should be made fresh.
2. Incubate at room temperature for 30-120 min with agitation.

3. Collect the eluant by centrifugation or using an empty spin column.
4. Wash the resin 2 additional times with PBS. Retain for analysis.

Samples Preparation for Gel Analysis

1. Add 600 μL methanol to 200 μL reaction mixture, vortex briefly.
2. Add 150 μL chloroform, vortex briefly.
3. Add 400 μL dH_2O , vortex briefly.
4. Centrifuge for 5 minutes at 13,000-20,000 x g, carefully remove upper aqueous layer without disturbing interface layer containing proteins. Note-upper aqueous layer may either be colorless or contain color depending on detection reagent.
5. Add 450 μL methanol, vortex briefly.
6. Centrifuge for 5 minutes at 13,000-20,000 x g to pellet protein. Carefully, remove and discard supernatant.
7. Add 450 μL methanol, vortex briefly. Repeat step 6.
8. Open the lid to microfuge tube and allow protein pellet to air-dry for at least 15 minutes.
9. Cap and store labeled sample at -20°C until ready for use.

Selected Publications

1. Yang Y., *et al.* (2013). Cleavable Trifunctional Biotin Reagents for Protein Labeling, Capture, and Release. *Chem. Commun.*, **49**:5366-06.