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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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HyLink™ BirA Biotin Labeling Kit,

100 µg*20

Cat no. LDG0021RC

Product Overview

Components	
GST-BirA (1 mg/mL)	40 µL, 2 vials
10X Reaction buffer	1 mL, 2 vials

Description

The HyLink™ BirA Biotin Labeling Kit for Avi-tag proteins provides a highly efficient, specific, and reproducible solution for biotinylating recombinant proteins or peptides containing Avi-tag or other enzymatic biotinylation tags such as Biotag™. This kit utilizes the biotin ligase enzyme BirA, which specifically and uniformly attaches biotin to a lysine residue within the Avi-tag sequence. Compared to traditional chemical biotinylation methods, this enzymatic approach offers site-specific labeling, minimizes variability between batches, and preserves the biological activity and structural integrity of the target protein.

Key Advantages

1. High Specificity and Consistency:
   BirA ligase precisely biotinylates the lysine residue within Avi-tags, avoiding random labeling and maintaining protein functionality.
2. High Efficiency and Reproducibility:
   Enzymatic biotinylation ensures uniform labeling with reduced batch-to-batch variation compared to chemical methods.
3. Wide Application:
   Suitable for various downstream applications including immunofluorescence staining, in situ hybridization (ISH), flow cytometry (FACS), binding assays, biopanning, and affinity purification.

Materials required but not provided

1. Disposable microcentrifuge tubes (1.5 or 2 mL).
2. 5 µL to 1000 µL adjustable single-channel micropipettes with disposable tips.
3. Timer
4. Vortex mixer
5. Incubator capable of maintaining temperature at 30±1℃ or 4±1℃.

Storage and Stability

1. Stored at -20°C. Avoid repeated freeze/thaw cycles.
2. Equilibrate the kit to room temperature before use.
3. The kit is stable for one year under proper storage conditions.

Procedure

Biotin conjugation protocol

1. For proteins conjugation:
   Use 10X Reaction buffer (e.g. Add 1 µL of 10x Reaction buffer for 9 µL of protein) to adjust the protein buffer condition.
2. Add GST-BirA (1 mg/mL) into the mixture containing target protein and 10X Reaction buffer (e.g. Add 1 µg of GST-BirA for 100 µg of protein). Mix gently by pipetting several times.

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www.leadgenebio.com
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3. Next, incubate at 30°C for 1 hour, or 4°C for 16 hours.
4. After incubating, use SpinDesalt Column (LDG0008RC) or dialysis method to remove excess biotin. The conjugates can be immediately used after desalting or dialysis.
5. Use Streptavidin-HRP or Streptavidin Gel-Shift Assay to assess the biotinylation efficiency, as described in the next section.

Streptavidin Gel-Shift Assay

1. The Streptavidin gel-shift assay provides a simple and effective method to evaluate the efficiency of protein biotinylation. By pre-incubating the biotinylated protein sample with streptavidin, a mobility shift can be observed on SDS-PAGE. A fully biotinylated protein will exhibit a complete shift in its migration pattern due to the binding of streptavidin.
2. For accurate interpretation, it is recommended to include two controls in the assay.
3. A biotinylated protein sample without Streptavidin incubation, to serve as a baseline reference.
4. Note: Residual free biotin may interfere with Streptavidin binding and affect the assay results.

Gel-Shift Assay Procedure

1. Determine the biotinylated protein concentration by appropriate methods (such as Bradford, Lowry, or A280).
2. Boil the 2 µg samples with denaturing dye in a dry heat block at 90°C for 3 minutes, then spin down.
3. Cool the samples to room temperature for 3 minutes.
4. To the tested sample add 2 µg of Streptavidin (not supply). Vortex briefly &Spin down.
5. Incubate for 5 minutes at room temperature. Then analyze both samples on SDS-PAGE.

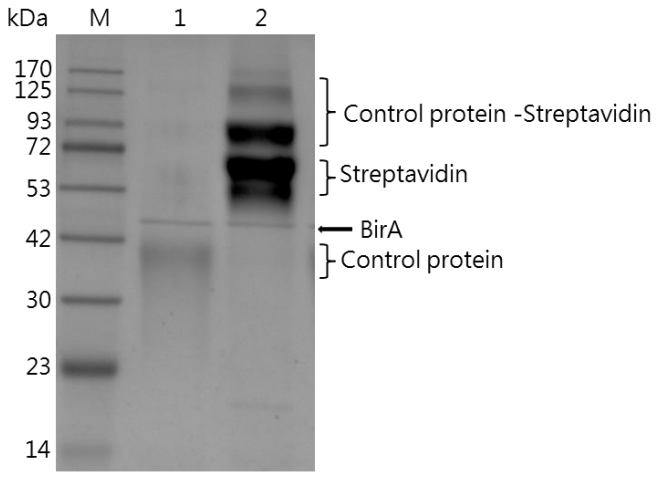

Figure 1. Streptavidin gel-shift assay. Protein biotinylation was analyzed using a Streptavidin gel shift assay. Lanes 1–2 contain equal amounts of control protein, analyzed by 4–12% SDS-PAGE followed by Coomassie blue staining.
Lane M: Protein Ladder
Lane1: Conjugated control protein, without Streptavidin
Lane2: Conjugated control protein, with Streptavidin

Important notes

Biotin labeling

1. Avi-tag protein concentrations of 0.25-2 mg/mL generally give optimal results.
2. Further adjustments to the BirA ligase-to-target protein ratio, incubation time, and temperature may be required to achieve optimal biotinylation, depending on the experimental results obtained for each target protein.

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