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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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HyLink™ BirA Biotin Labeling Kit

For biotinylation of up to 8 mg target protein

Cat no. LDG0021RC

Product Overview

Components	
GST-BirA (lyophilized)	40 µg, 1 vial
Reconstitution buffer	0.2 mL, 1 vial
10X Reaction buffer	1.5 mL, 1 vial
SpinDesalt Column(LDG0008RC)	16 pcs

Description

The HyLink™ BirA Biotin Labeling Kit for Avi-tagged proteins offers a reliable, efficient, and site-specific method for biotinylating recombinant proteins or peptides containing Avi-tag. This kit utilizes the BirA biotin ligase, which catalyzes the precise attachment of biotin to a specific lysine within the Avi-tag. Unlike conventional chemical biotinylation methods, this enzymatic labeling ensures consistency, reduces batch-to-batch variation, and maintains the native structure and function 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.

Storage and Stability

1. Stored at -20°C. Avoid repeated freeze/thaw cycles after reconstituted.
2. The kit is stable for one year under proper storage conditions.
3. The reconstituted GST-BirA is stable for 6 months under proper storage conditions.

Procedure

*Reconstitute GST-BirA lyophilized powder with 40 µL reconstitution buffer before used.
*It is recommended to aliquot the reconstituted GST-BirA to avoid repeated freeze-thaw cycles.

Biotin Conjugation Protocol

1. Prepare a reaction system according to the table below (e.g. Add 1 µL of GST-BirA for 100 µg of protein). After gently pipetting and mixing, incubate at 4°C for 16 hours.

Components	Volume (µL)	Final Concentration
GST-BirA	1	-
Protein (100 µg)	a	-
10X Reaction buffer	b/10	1X
Total volume	b	-

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* Note:

a. A protein concentration of at least 0.5 mg/mL is recommended.

b. Optimal biotinylation may require adjusting the BirA ligase-to-target protein ratio (1:50 to 1:200, with 1:100 recommended), incubation time, and temperature based on experimental results for each target protein.

2. After incubating, use SpinDesalt Column (LDG0008RC) or dialysis method to remove excess biotin.
3. The conjugates can be immediately used after desalting or dialysis. If needed, use glutathione magnetic beads to remove the GST-BirA.
4. Use streptavidin-HRP or streptavidin gel-shift assay to assess the biotinylation efficiency, as described in the next section.

SpinDesalt Column Protocol

1. Prepare a SpinDesalt Column by breaking off the bottom closure and placing the column into a microcentrifuge tube.
2. Centrifuge the column at 1,000 × g for 1 minute, discard the storage buffer and return column to the same microcentrifuge tubes.
3. Adding 0.25 mL of PBS to the top of the resin bed and centrifuging at 1,000 × g for 1 minute. Discard the flowthrough and repeat this step 3 times.
4. Place the column into a new microcentrifuge tube and apply approximately 0.1-0.25 mL of the conjugates directly onto the resin bed. Centrifuge the column at 1,000 × g for 1 minute.
5. The collected flowthrough solution is purified conjugates.

   * Each SpinDesalt column can process a minimum of 250 µg of total protein.

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 of results, it is recommended to include appropriate controls: A biotinylated protein sample without streptavidin incubation, serving as a baseline control to assess unshifted protein mobility.

   * Note: Residual free biotin can compete with biotinylated protein for streptavidin binding and affect assay accuracy. Remove excess biotin before the assay (e.g., by dialysis or desalting).

Gel-Shift Assay Procedure

1. Determine the concentration of biotinylated protein by appropriate methods (e.g., Bradford, Lowry, or A280 measurement).
2. Mix 2 µg of the protein sample with SDS-PAGE protein loading buffer, heat at 95 °C for 5 minutes, then spin down.
3. Cool the samples to room temperature for 5 minutes.
4. Add 2 µg of streptavidin (not provided) to the tested sample. Vortex briefly and spin down.
5. Incubate the samples at room temperature for 5 minutes, then analyze by SDS-PAGE.

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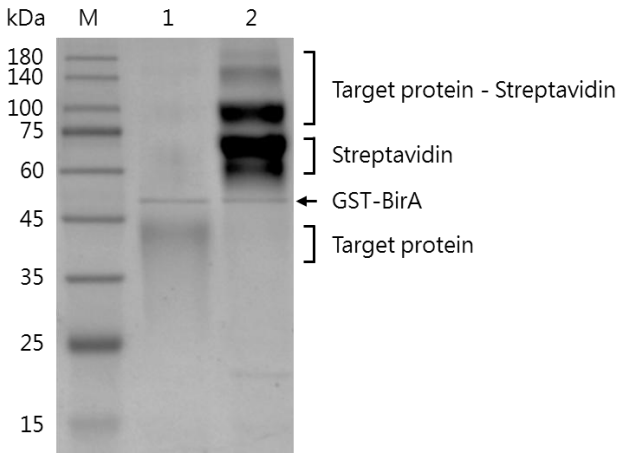

Figure 1. Streptavidin gel-shift assay.
Protein biotinylation was analyzed using a streptavidin gel-shift assay. Lane 1–2 contain equal amounts of control protein, analyzed by 15% SDS-PAGE followed by Coomassie blue staining.
Lane M: Protein Ladder
Lane 1: Conjugated target protein, without streptavidin
Lane 2: Conjugated target protein, with streptavidin

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