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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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HyLink™ Biotin Labeling Kit (SpinDesalt Column), 100 µg*10

Cat no. LDG0007RC

Product Overview

Components	
Biotin	1 mg, 1 vial
Biotin reconstitution buffer	300 µL, 1 vial
10X Modifier	300 µL, 1 vial
PBS	10 mL, 1 vial
SpinDesalt Column (LDG0008RC)	10 vials

Description

Biotin is a widely used and powerful tool for research due to the specific and high affinity with streptavidin/avidin. Antibody or protein conjugated with several biotin molecules can also amplify the detection signal through streptavidin-conjugated molecule such as streptavidin-HRP, streptavidin-FITC, etc. Biotinylated antibody or protein can be used in various applications including ELISA, WB, IHC, IFA and FACS. Leadgene HyLink™ Biotin Labeling Kit (SpinDesalt Column) is designed for biotinylation of antibody or protein (100 µg-1 mg). It provides a rapid and easy process with high efficiency to conjugate biotin to antibody or protein.

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 37± 1℃.

Storage and Stability

(1) Stored at -20°C except for **SpinDesalt Column** stored at 4°C. Avoid repeated freeze/thaw cycles.
(2) Equilibrate kit to room temperature before use.
(3) The kit is stable for one year under proper storage conditions.

Procedure

Biotin conjugation protocol

- **For antibody conjugation**

(1) Dissolve the 1 mg **Biotin** with 200 µL **Biotin reconstitution buffer** to a concentration of 5 mg/mL.
(2) Dissolve antibody in PBS **(Refer to Important notes point 1 before dissolving)** or other buffers that do not contain amine, Tris or glycerol. Use **10X Modifier** (e.g. Add 1 µL of 10X Modifier for 9 µL of antibody) to adjust the antibody buffer condition.
(3) Make sure all buffers are well dissolved before using. If not, please vortex the vial to make salts dissolved.
(4) Add **Biotin** into the mixture that containing antibody and 10X Modifier (e.g. **Add 20 µL of Biotin for 100 µg of antibody**). Mix gently by pipetting several times.
(5) Next, incubate in the dark at room temperature for 1 hour, then move to 4°C for 2 hours.
(6) After incubating, using **SpinDesalt Column** to remove excess biotin **(Refer to Important notes point 2 before using column)**. The conjugates can be used after desalting.

- **For protein conjugation**, the amount of protein can be calculated by formula below:

$$\text{Quantities of protein} = \text{Biotin conjugation (e.g. 1 mg)} \times \frac{\text{M.W. (KDa) protein}}{\text{150 kDa IgG}}$$

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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 the purified conjugates.

Important notes

1. Biotin labeling

(1) Antibody concentrations of 1-2 mg/mL generally give optimal results. Recommended amount and volume of antibody for optimal results.

Kit size	Antibody amount	Reaction volume
100 µg x 10	0.1 mg-2 mg	100-250 µL

(2) Common non-buffering salts (e.g. sodium chloride) have no effect on conjugation efficiency. Avoid buffer component that contains primary amine (e.g. amino acid or ethanolamine) and thiols (e.g. 2-Mercaptoethanol or DTT).
Components that have on effect or little effect on labeling reaction:
-up to 50 mM Tris
-up to 50 mM HEPES
-up to 10% glycerol
-up to 0.02% sodium azide

2. SpinDesalt Column

(1) For conjugates < 0.1 mL, please use PBS to adjust the volume to at least 0.1 mL to increase the recovery rate of SpinDesalt Column.

(2) The recovery rate of the SpinDesalt Column is related to the type of protein and other biomolecules, usually exceeding 85%. Increasing the sample concentration or volume can improve the recovery rate.

(3) After completing the conjugation, the resin bed in the column can be temporarily stored in PBS.

Disclaimer

This product is for research use only and is not intended for diagnostic use.