

PRODUCT INFORMATION

Biotin Conjugation Kit (SpinDesalt Column)

v. 250301

Catalog number	C08013-1000												
Package	100 µg X 10												
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. Biotin Labeling Kit (SpinDesalt Column) is designed for biotinylation of a small quantity (100 µg-200 µg) of antibody or protein. It provides a rapid and easy process with high efficiency to conjugate biotin to antibody or protein.												
Component	<table> <tr> <td></td><td>100 µg x 10</td></tr> <tr> <td>Biotin</td><td>1 mg, 1 vial</td></tr> <tr> <td>Biotin reconstitution buffer</td><td>300 µL, 1 vial</td></tr> <tr> <td>10X Modifier</td><td>300 µL, 1 vial</td></tr> <tr> <td>PBS</td><td>10 mL, 1 vial</td></tr> <tr> <td>SpinDesalt Column(C08012-K01)</td><td>10 vials</td></tr> </table>		100 µg x 10	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(C08012-K01)	10 vials
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SpinDesalt Column(C08012-K01)	10 vials												
Stability & Storage	(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.												
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°C.												

Biotin conjugation protocol

• For antibody conjugation

1. Dissolve the 1 mg **Biotin** with 200 μ L **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 μ L of **10X Modifier** for 9 μ L 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 μ L of Biotin for 100 μ g 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.

Procedure

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

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

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 $\times$ 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 $\times$ 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 $\times$ g for 1 minute.
- (5) The collected flowthrough solution is the purified conjugates.

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 an 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

Important notes

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.

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