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LEADGENE BIOMEDICAL, INC.

# Product Information & Manual

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

**Cat no. LDG0010RC**

### Product Overview

**Package component**

| Package | (100 µg x 10) | Storage |
|---|---|---|
| FITC | 10 vials | -20℃ |
| 10X Modifier | 1 vial | -20℃ |
| 10X Quencher | 1 vial | -20℃ |
| SpinDesalt Column (LDG0008RC) | 10 vials | 4℃ |

### Description

Fluorescein isothiocyanate (FITC) is a widely used fluorescent dye with an excitation peak at 491 nm and an emission peak at 516 nm. Leadgene HyLink™ FITC Labeling Kit (SpinDesalt Column) is designed for small scale FITC conjugation. It provides a rapid and easy process with high efficiency to conjugate antibodies or protein to FITC. The total process completed in 3 hours and less than 30 minutes hands-on time.

### Procedure

(1) Equilibrate reagent to room temperature before using. Make sure all buffers are well dissolved. If not, please vortex the vial to make salts dissolved.

(2) Dissolve antibody in PBS or other buffer that do not contain amine, tris, NaN3 or glycerol. Add 10X Modifier to antibody (e.g. 1 µL of 10X Modifier for 9 µL of antibody).

(3) Spin down the vial of FITC before using.

(4) Open the cap of the vial of FITC and pipette antibody into the vial. Mix gently by pipetting several times until FITC dye is well dissolved.

(5) Cover the cap on the vial and spin down the vial. Incubate in the dark at room temperature for 2 hours.

(6) Method 1:
Using SpinDesalt Column to remove unconjugated FITC. Please refer to the protocol of SpinDesalt Column, catalogue no. LDG0008RC. Collect labeled antibody and stabilize with 1% bovine serum albumin or another stabilizer.
Method 2:
Add 10X Quencher to Ab-Fluorescent mixture. Incubate in the dark at room temperature for 30 minutes. Stabilized with 1% bovine serum albumin or another stabilizer.

(7) Store the labeled protein protected from light.

(8) For protein conjugation, it can be calculated by formula below:
Quantities of protein = quantities of kit (e.g. 100 µg) x (M.W.of target protein)/(150000 (M.W.of IgG)

### Important notes

(1) Antibody concentrations of 0.5-2 mg/mL generally give optimal results.

| Kit size | Antibody amount | Reaction volume |
|---|---|---|
| 100 µg x 10 | 50-200 µg | 40-200 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. mercaptoethanol or DTT).



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**Determine of DOL (degree of labeling)**

(1) Calculate concentration of protein:

$$\text{Portein concentration (M)} = \frac{A_{280}-(A_{max}\times CF)}{\varepsilon_{protein}} \times \text{Dilution factor}$$

* CF : (Correction factor) = 0.3

* $\varepsilon_{protein}$: protein molar extinction coefficient.

(The molar extinction coefficient of IgG is 210000 $M^{-1}cm^{-1}$.)

(2) Calculate DOL:

$$\text{DOL} = \frac{A_{max} \text{ of labeled protein}}{\varepsilon_{FITC}\times \text{Protein concentration (M)}} \times \text{Dilution factor}$$

* $\varepsilon_{FITC}$ = 70000 $M^{-1}cm^{-1}$.)

**Disclaimer**

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

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## SpinDesalt Column

**Cat no. LDG0008RC**

### Product Overview

#### Package

SpinDesalt Column, 0.5 mL, 5 columns

#### Introduction

Leadgene SpinDesalt Column is a convenient and rapid product that is suitable for desalting, buffer exchange, or removal of small molecules from proteins or other macromolecular samples with a molecular weight larger than 5 kDa. The SpinDesalt Column is filled with 0.5 mL of Smartdex G-25, which efficiently separates proteins from small molecules (such as salt and other small molecules like biotin).

#### Storage

SpinDesalt Column should be store at 2-8℃.

### Procedure

#### Preparation of the equilibration buffer

The equilibration buffer can be chosen according to the buffer you want to replace. It's recommended to filter it with a 0.22 μm or 0.45 μm membrane before using the buffer.

#### Preparation of the sample

It is recommended to centrifuge the sample or filter it with a 0.22 μm or 0.45 μm membrane before loading, to reduce the impact of contaminant.

#### SpinDesalt Column protocol

(1) Prepare a SpinDesalt Column by breaking off the bottom closure and placing the column into a 2 mL collection tube.

(2) Centrifuge the column at 1,000 × g for 1 minute, discard the storage buffer and return column to the same collection tube.

(3) Adding 0.25 mL of the equilibration buffer 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 1.5 mL collection tube and apply approximately 0.1-0.2 mL of the sample directly onto the resin bed. Centrifuge the column at 1,000 × g for 1 minute.

(5) The collected flowthrough solution is the purified sample.

#### Important notes

(1) Please break off the bottom closure before using the column.

(2) Please centrifuge the column at 1,000 x g.

(3) Each column can process a 100-200 μL sample at a time.

(4) 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.

(5) If sample < 0.1 mL, please use the equilibration buffer to adjust the volume to at least 0.1 mL to increase the recovery rate.

(6) The resin bed of SpinDesalt Column can be temporarily stored in the equilibration buffer.



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