LEADGENE
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:

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

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

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

Dye	Amax / Emission	CF (Correction factor)	Extinction coefficient (ε) $M^{-1}cm^{-1}$
FITC	491 / 516	0.3	70000

(2) Calculate DOL:

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

* ε_{FITC} = 70000 $M^{-1}cm^{-1}$.

Disclaimer

For Research Use or Further Manufacturing Only.

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