MCE® MedChemExpress

Product Data Sheet

Inhibitors • Screening Libraries • Proteins

Vari Fluor 488 SE

Cat. No.: HY-D1801

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light
* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)

SOLVENT & SOLUBILITY

In Vitro	DMSO : 100 mg/mL (Need ultrasonic)
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (Infinity mM); Clear solution 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (Infinity mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Vari Fluor 488 SE (VF 488 SE) is a dye marker of the Vari Fluor SE series (Ex/Em=488 nm/513 nm). The Vari Fluor SE series of dyes are a class of fluorescent dyes containing NHS ester groups used to label free amines (-NHX) on antibodies, proteins, peptides, amine-modified oligonucleotides and other biomolecules.

In Vitro

Protocol

1.Protein Preperation
1) In order to obtain the best labeling effect, please prepare the protein (antibody) concentration as 2mg/mL.
2) The pH value of protein solution shall be 8.5±0.5. If the pH is lower than 8.0, 1 M sodium bicarbonate shall be used for adjustment.
3) If the protein concentration is lower than 2 mg/mL, the labeling efficiency will be greatly reduced. In order to obtain the best labeling efficiency, it is recommended that the final protein concentration range is 2-10 mg/mL.
4) The protein must be in the buffer without primary amine (such as Tris or glycine) and ammonium ion, otherwise the labeling efficiency will be affected.
2.Dye Preparation�Example for VF 488 SE�
Add anhydrous DMSO into the vial of VF 488 SE to make a 10 mg/mL stock solution. Mix well by pipetting or vortex.
3.Calculation of dye dosage
The amount of VF 488 SE required for reaction depends on the amount of protein to be labeled, and the optimal molar ratio of VF 488 SE to protein is about 10.
Example: assuming the required marker protein is 500 μL 2 mg/mL IgG (MW=150,000), use 100 μL DMSO dissolve 1 mg VF 488 SE, the required VF 488 SE volume is 5.05 μL, and the detailed calculation process is as follows:
1) mmol (IgG) = mg/mL (IgG)×mL (IgG) / MW (IgG) =2 mg/mL × 0.5 mL / 150,000 mg/mmol= 6.7×10^{-6} mmol

2) mmol (VF 488 SE) = mmol (IgG)× 10 = 6.7×10^{-6} mmol×10 = 6.7×10^{-5} mmol
3) µL (VF 488 SE) = mmol (VF 488 SE)×MW (VF 488 SE) / mg/µL (VF 488 SE) = 6.7×10^{-5} mmol ×834 mg/mmol / 0.01 mg/µL = 5.6 µL (VF 488 SE)

4.Run conjugation reaction

1) A good volume of freshly prepared 10 mg/mL VF 488 SE is slowly added to 0.5 mL protein sample.
In solution, gently shake to mix, then centrifuge briefly to collect the sample at the bottom of the reaction tube. Don't mix well to prevent protein samples from denaturation and inactivation.

2) The reaction tubules were placed in a dark place and incubated gently at room temperature for 60 minutes at intervals.For 10-15 minutes, gently reverse the reaction tubules several times to fully mix the two reactants and raise the bar efficiency.

5.Purify the conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1�Prepare Sephadex G-25 column according to the manufacture instruction.
2�Load the reaction mixture (From "Run conjugation reaction") to the top of the Sephadex G-25 column.
3�Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
4�Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

Note

1.CY dyes is sensitive to light and humidity. Immediately add CY dyesr solution and discard the unused part.
2.Low concentrations of sodium azide (≤3 mM or 0.02%) or thiomersal (≤0.02 mM or 0.01%) did not significantly interfere with protein labeling; However, 20-50% glycerol will reduce labeling efficiency.
3.Avoid buffering with primary amines (e.g., Tris, glycine) or ammonium ions,It compete with labeled proteins.
4.This product is only for scientific research by professionals, and shall not be used in clinical diagnosis or treatment, food or medicine.
5.For your safety and health, please wear lab coat and disposable gloves.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Nanda JS, et al. Labeling a protein with fluorophores using NHS ester derivitization. Methods Enzymol. 2014;536:87-94.

Caution: Product has not been fully validated for medical applications. For research use only.

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