Product Data Sheet

Product Name: AF532 NHS Ester
Cat. No.: GC80399

Chemical Properties

Cas. No.			
Formula		M.Wt	
Solubility		Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

AF532 NHS Ester

Background

AF 532 NHS ester is a fluorescent dye AF 532 derivative exhibits high fluorescence quantum yield and high photostability. AF 532 NHS ester can form an ester bond with ammonia via the NHS group,

and can be used to label proteins, amine-modified oligonucleotides, and other primary amines (R-NH2) containing amine molecules (Ex/Em = 530/555 nm).

In Vitro, Guide (The following is our recommended protocol. This protocol is only a guide and should be modified according to your specific needs). Stock Solution Preparation 1. Protein Preparation To obtain the best labeling effect, please prepare the protein (antibody) concentration to 2 mg/mL. 1.1 The pH value of the protein solution should be 8.5 ± 0.5. If the pH is lower than 8.0, adjust it with 1 M sodium bicarbonate. 1.2 If the protein concentration is lower than 2 mg/mL, the labeling efficiency will be greatly reduced. To obtain the best labeling efficiency, it is recommended that the final protein concentration range be 2-10 mg/mL. 1.3 The protein must be in a buffer free of primary amines (such as Tris or glycine) and ammonium ions, otherwise the labeling efficiency will be affected. 2. Dye Preparation Dilute the dye with anhydrous DMSO to prepare a clear stock solution of 10 mg/mL. 3. Calculation of Dye Amount The amount of dye required for the reaction depends on the amount of protein to be labeled. The optimal molar ratio of dye to protein is approximately 10:1. Note: We recommend using a dye:protein molar ratio of 10:1. If the concentration is too low or too high, the ratio can be adjusted to 5:1, 15:1, or 20:1. 4. Running the Coupling Reaction 1) Slowly add the calculated volume of freshly prepared 10 mg/mL dye to 0.5 mL of protein sample solution, gently shake to mix, and then briefly centrifuge to collect the sample at the bottom of the reaction tube. Avoid vigorous mixing to prevent denaturation and inactivation of the protein sample. 2) Place the reaction tube in a dark place and incubate gently with shaking for 60 minutes at room temperature. Every 10-15 minutes, gently invert the reaction tube several times to thoroughly mix the reactants and improve labeling efficiency. 5. Purification of Conjugates The following protocol is an example of purifying dye-protein conjugates using a Labelling Kits Centrifugation-Based Rapid Desalting Column (5KD). 5.1 Prepare the desalting column according to the instructions. 5.2 Load the reaction mixture onto the top of the desalting column. 5.3 Once the sample has run below the resin surface, immediately add PBS (pH 7.2-7.4). 5.4 Add more PBS (pH 7.2-7.4) to the target sample to complete column purification. Collect the fraction containing the desired dye-protein conjugate.

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