Data Sheet (Cat.No.T209512)

TargetMol

Cy5.5 acetate

Chemical Properties

CAS No. :

Formula: C43H48N2O16S4

Molecular Weight:

Storage: Keep away from direct sunlight
Store at -20°C
Actual storage temperature shall be subject to the COA.

Biological Description

Description	Cy5.5 acetate is a CY dye, where CY stands for cyanine—an organic compound comprised of two nitrogen atoms linked with an odd number of methine units. Cyanine compounds are known for their long wavelengths, adjustable absorption and emission, high extinction coefficients, good water solubility, and relatively simple synthesis. CY dyes are frequently used for labeling proteins, antibodies, and small molecules. For protein and antibody labeling, the attachment can be achieved through a straightforward mixing reaction. Below, we outline a labeling method for proteins and antibodies, which may serve as a useful reference.
Targets(IC50)	Others
In vitro	Protein (Antibody) Preparation: To achieve optimal labeling efficiency, adjust the protein concentration to 2 mg/mL. Ensure the solution has a pH of 8.5±0.5; if it's below 8.0, adjust with 1 M sodium bicarbonate. The labeling efficiency significantly drops if the concentration falls below 2 mg/mL, so aim for a final range of 2–10 mg/mL. Proteins must be in buffers free of primary amines (e.g., Tris or glycine) and ammonium ions to avoid efficiency issues. Dye Preparation: Dilute CY dye with anhydrous DMSO to a 10 mM stock solution, ensuring it's well-mixed through a glass pipette or vortex. Store the stock in aliquots at -20 °C to -80 °C, shielded from light. Activate with conjugate solution (500 μg/mL) before proceeding with labeling experiments. Calculating Dye Working Solution Volume: The amount of CY dye needed depends on the protein to be labeled, with an optimal molar ratio of CY dye to protein being around 10. For example, if labeling 500 μL of 2 mg/mL IgG (MW=150,000) using 100 μL DMSO to dissolve 1 mg of CY dye, you will need 3.95 μL of CY. Detailed calculations (taking CY3-NHS ester as an example) are 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 (CY3-NHS ester) = mmol (IgG) × 10 = 6.7×10^-6 mmol × 10 = 6.7×10^-5 mmol 3) μL (CY3-NHS ester) = mmol (CY3-NHS ester) × MW (CY3-NHS ester) / mg/μL (CY3-NHS ester) = 6.7×10^-5 mmol × 590.15 mg/mmol / 0.01 mg/μL = 3.95 μL (CY3-NHS ester)

In vitro	Method of Use: Labeling Reaction: Activate 10 mM CY dye (approximately 10 μL of stock plus 50 μL of 500 μg/mL conjugate solution), then gently add it to 0.5 mL of protein sample, mixing gently to prevent denaturation. Briefly centrifuge to collect the sample at the tube's bottom. Place the reaction tube in a dark environment and incubate at room temperature for 60 minutes, gently inverting it every 10–15 minutes to enhance mixing and labeling efficiency. Protein Purification and Desalting: Use a Sephadex G-25 column to purify dye-protein conjugates. Prepare the column according to the manufacturer's instructions. Load the reaction mixture onto the column and, once the sample reaches just below the resin's surface, add PBS (pH 7.2-7.4). Complete column purification by eluting with additional PBS (pH 7.2-7.4) to collect desired dye-protein conjugates. The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands.
In vivo	Cy5.5-labeled factor VIIa has been designed for tumor imaging. This targeted protein-bound Cy5.5 specifically localizes to tumor xenografts for at least 14 days, while unbound Cy5.5 does not localize to any xenografts or organs. This method of imaging anti-tissue factor in tumor VEC can be used to detect primary tumors and metastases, as well as to monitor treatment responses in vivo. Additionally, a pH/temperature-sensitive magnetic nanogel combined with Cy5.5-labeled lactoferrin (Cy5.5-Lf-MPNA nanogel) has been developed as a promising contrast agent for preoperative MRI and intraoperative fluorescence imaging of gliomas.