Product Data Sheet

Product Name: Sulfo-QSY21-NHS DIEA
Cat. No.: GC81856

Chemical Properties

Cas. No.

Formula	$C_{53}H_{57}N_5O_{13}S_3$	M.Wt	1068.24
Solubility		Storage	Store at -20°C, sealed storage, away from moisture and light

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

Background

Sulfo-QSY21-NHS DIEA is an N-hydroxysuccinimide ester derivative fluorescent quencher that reduces the fluorescence intensity of paired fluorophores via proximity effect and relieves quenching after linker extension induced by mechanical force. Sulfo-QSY21-NHS DIEA can label streptavidin through NHS ester bioconjugation and be integrated into fluorescence-based molecular tension sensors to map the mechanical forces of cell surface receptors. Sulfo-QSY21-NHS DIEA also serves as a quencher for cysteine cathepsin-targeted quenched fluorescent substrate probes, and is widely used in studies related to breast cancer, colorectal cancer and lung adenocarcinoma [1] [2].

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

In Vitro, A: QSY 21 NHS Ester Labeling of Streptavidin [1] Method 1 (Size-exclusion purification, target labeling ratio ≈ 1): 1. Dissolve 300 μg of recombinant streptavidin in 150 μl of 1× PBS (10 mM phosphate buffer, 137 mM NaCl, pH 7.4). 2. Add 15 μl of 1 M sodium bicarbonate and a 20-fold molar excess of QSY 21 NHS ester (Invitrogen). 3. Incubate for 60 min on a rotating platform at room temperature (23 °C). 4. Perform size-exclusion chromatography purification using Bio-Gel P4 resin (Bio-Rad) swollen with 1× PBS. 5. Characterize the product by MALDI-TOF and UV-Vis absorption spectroscopy; the labeling ratio determined by UV-Vis is 0.8. Method 2 (Dialysis purification, adjustable labeling ratio): 1. Prepare a solution of recombinant streptavidin at 1 mg/mL in 1× PBS. 2. Add an excess of QSY 21 NHS ester (5-fold or 20-fold molar excess). 3. Incubate at room temperature for 60 min, inverting the mixture every 15 min to mix. 4. Purify using a Slide-a-Lyzer Mini dialysis column (Thermo Fisher, molecular weight cutoff 3,500 g/mol) according to the manufacturer's instructions, dialyzing twice against 2 L of 1× PBS for 30 min each time. 5. Characterize the product by MALDI-TOF and absorption spectroscopy. A 5-fold molar excess yields a labeling ratio of approximately 0.9-1.1; a 20-fold molar excess yields a labeling ratio of approximately 2. B: In Vitro Enzyme Kinetics Assays of Cathepsin Probes Containing the sulfo-QSY21 Quencher[2] 1. Recombinant cysteine cathepsins (Cat. L, B, S, V, K) are commercially obtained. Prior to use, the enzyme concentration is confirmed by active-site titration using the irreversible inhibitor ZFK-chloromethyl ketone and the substrates Z-VVR-AMC (for Cat. L/S/B/V) or Z-KR-AMC (for Cat. K). 2. Primary screening: Fixed concentrations of each quenched substrate probe (containing Cy5 chromophore and sulfo-QSY21 quencher, nCQ/nQC, n=2,4,6) are dissolved with proteases in 50 mM citrate buffer (pH 5.5, 5 mM DTT, 0.1% Triton X, 0.5% CHAPS), followed by incubation in a black opaque 96-well plate at 37 °C. The Cy5 fluorescence (640/670 nm) growth curve is monitored using a Biotek microplate reader. 3. Determination of kinetic parameters: Each enzyme is used at a final concentration of 5 nM and incubated with serially diluted quenched substrates (final concentrations ranging from 10 μM to 0 μM, in triplicate). The Cy5 fluorescence growth rate is read from the linear segment of the conversion curve. 4. KM, kcat and kcat/KM are obtained by fitting data to the Michaelis-Menten equation using GraphPad Prism software. 5. Results: All probes are cleaved by Cat. L, V, S, and K (but not by Cat. B); kcat/KM increases with the increase of linker length, reaching the highest value when n=6; KM values are similar across all probes, and the differences in kcat/KM arise from variations in kcat. C: Live-cell Imaging Assay with Probes Containing sulfo-QSY21 Quencher[2] 1. RAW264.7 cells are seeded in 35×10 mm glass-bottom dishes at a density of approximately 1×104 cells and cultured for 24 h. 2. The culture medium (DMEM + 10% FBS + 100

units/mL penicillin + 100 μg/mL streptomycin) is replaced with 1 mL of DMEM containing 1 μM of the specified quencher probe. 3. The cells are incubated for 30 min in a humidified environment at 37 °C with 5% CO2. 4. The cells are washed once with PBS, followed by incubation in fresh medium containing 60 nM Lysotracker for 30 min. 5. The cells are washed with PBS and then incubated in DMEM containing 5 μg/mL Hoechst 33342 for 10 min. 6. The cells are washed three times with PBS, covered with PBS, and images of Cy5, Hoechst, and Lysotracker Red channels are acquired using a Zeiss Axiovert 200 M confocal microscope with a 60× objective, followed by processing with ImageJ. 7. Results: All probes produce strong fluorescence and show high colocalization with Lysotracker Red (lysosomal localization), and the labeling intensities of nCQ and nQC probes are similar. D: Live-Cell Mechanical Imaging Experiment of EGFR Tension Sensor Based on QSY 21[1] Sensor Surface Preparation: 1. Glass coverslips are sonicated in Nanopure water for 10 min → etched in piranha solution (3:1 H2SO4:H2O2) for 10 min → rinsed with water 6 times → soaked in ethanol 3 times → incubated in 1% APTES ethanol solution for 1 h → washed with ethanol, dried with nitrogen gas → baked at 100 °C for 10 min. 2. Incubated with NHS-biotin (2 mg/mL DMSO) at room temperature overnight → washed with ethanol, dried with nitrogen gas → rinsed 3 times with 1× PBS → blocked with BSA (100 μg/μl) for 30 min → rinsed 3 times with PBS. 3. Incubated with QSY 21-labeled streptavidin (1 μg/mL) at room temperature for 45 min → rinsed 3 times with PBS. 4. Incubated with biotinylated EGF-PEG-Alexa Fluor 647 (1 μg/mL) at room temperature for 45 min → rinsed 3 times with PBS, and used on the same day. Cell Experiments: 1. HCC1143 cells are cultured in RPMI 1640 medium (supplemented with 10% FBS, 9.9 mM HEPES, 1 mM sodium pyruvate, 2.1 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin) at 37 °C under 5% CO2, followed by serum starvation for approximately 18 h. 2. Cells are seeded onto the sensor surface and cultured at 37 °C for 20-30 min until cell spreading is achieved. 3. Live-cell time-lapse imaging is performed using a Nikon Eclipse Ti TIRF microscope (640 nm laser, 100× NA 1.49 objective lens), with the temperature maintained at 37 °C. 4. Results: Transient localized fluorescence enhancement (mechanical events) is observed, with spots of diffraction-limited size and a duration mostly <30 s; treatment with latrunculin B (an actin inhibitor) reduces the signal by 70%; colocalization with CLC-eGFP confirms association with clathrin-mediated endocytosis; the peak force of the EGF-PEG24 sensor is approximately 4 pN.

References:

[1]. Stabley DR, et al. Visualizing mechanical tension across membrane receptors with a fluorescent sensor. Nature methods. 2011 Oct 30;9(1):64-7.
[2]. Ofori LO, et al. Design of Protease Activated Optical Contrast Agents That Exploit a Latent

Product Data Sheet

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