

Product Data Sheet

Product Name: IRDye 700DX

Cat. No.: GC71797

Chemical Properties

Cas. No. 916821-46-0

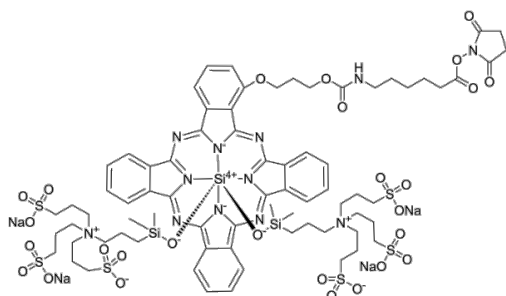
Formula $C_{74}H_{96}N_{12}Na_4O_{27}S_6Si_3$ M.Wt 1954.22

Solubility Storage -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



Protocol

IRDye 700DX-based single molecule imaging for cells

This experimental protocol is derived from the literature. Please make modifications according to your specific requirements.

1. Preparation of IRDye 700DX-biotin-transferrin conjugates:

(1) biotin-transferrin was dissolved in 0.1M $NaHCO_3$ pH 8.3 at a final concentration of 1mg/ml.

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Product Data Sheet

(2) IRDye 700DX and NHS (DMSO 10mg/mL, $\leq 5\%$ final volume) were added at a molar ratio of 1:3 and kept in the dark at room temperature for 60min.

(3) PD-10 desalting column was used to remove free dye and collect the first peak. Measure A_{280}/A_{689} , and calculate labeling degree 1.5-2.5 dye/protein is preferred.

(4) The samples were stored at -80°C in the dark to avoid repeated freezing and thawing.

2. Single molecule anchoring on the slide surface (for system correction):

(1) The new dishes were washed for 30min, rinsed with pure water, and blow-dried with nitrogen.

(2) 1mg/ml BSA-biotin was added and the cells were left at room temperature for 10min.

(3) Wash with TE buffer 3 times. 0.2mg/ml streptavidin was added for 10min.

(4) Then gently wash, 0.05nM IRDye 700DX-biotin-transferrin (diluted with TE) was added and the mixture was maintained at room temperature for 10min.

(5) Free conjugates were washed off with TE buffer and used immediately for imaging, or stored at 4°C in the dark for ≤ 2 h.

3. Single molecule markers of cell samples:

(1) MCF-7 cells grow to 30-40% confluence in a 35-mm glass bottom dish (too high density will increase the background).

(2) The transferrin receptor was switched to serum-free RPMI 1640 and incubated at 37°C for more than 18h.

(3) Pre-cooling at 4°C and adding 0.05nM IRDye 700DX-biotin-transferrin (0.1nM total biotin-transferrin containing 50-fold unlabeled biotin-transferrin as a competitive dilution to ensure single molecule binding) at 4°C for 1h.

(3) Wash 3 times with cold PBS; The cells were fixed with 4% PFA for 10min at room temperature.

(5) Replace with buffer solution, immediately on the machine, or stored at 4°C in the dark for ≤ 4 h.

4. Single molecule imaging acquisition:

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- (1) The TIRF Angle was optimized to irradiate the laser at a depth of 100-150nm. Laser power 1-2W/cm² ($\approx$ 3-5mW incident).
- (2) EMCCD gain 300, exposure 50-100ms, continuous acquisition of 1000-3000 frames (10Hz).
- (3) The 695nm and 780nm far red channels (for background subtraction and polychromatic expansion) were recorded.
- (4) A "dye-free" blank dish was added to each batch of experiments, and the mean value of the blank + 5 σ was used as the lower limit of detection when setting the threshold.

5. Precautions

- (1) Dark operation: IRDye 700DX is light sensitive, and all operations should be carried out in dark conditions.
- (2) Laser safety: 690nm laser is potentially harmful to the eyes, so wear goggles when operating.
- (3) Dye ratio control: too much dye binding may affect antibody activity. 2-4 dyes per antibody binding is recommended.
- (4) Cell state monitoring: the morphological changes of cells should be observed before and after light exposure to avoid non-specific killing caused by excessive light exposure.

References:

[1] Tynan C J, Clarke D T, Coles B C, et al. Multicolour single molecule imaging in cells with near infra-red dyes[J]. PloS one, 2012, 7(4): e36265.

Background

IRDye 700DX is a near-infrared (NIR) photosensitive dye with a maximum absorption wavelength of 689nm and an emission maximum of 702nm^[1]. The IRDye 700DX-antibody conjugates selectively accumulate within the target tumor and exhibit phototoxicity by binding to target cells, with minimal toxicity to normal tissues^[2]. IRDye 700DX-antibody conjugates accumulating in a perivascular region in a tumor can destroy the corresponding tissue and increase the vascular permeability in the tumor^[3]. IRDye 700DX has been widely used in combination with monoclonal antibodies to develop novel photoimmunotherapy to kill cancer cells^[4].

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References:

- [1] Tynan C J, Clarke D T, Coles B C, et al. Multicolour single molecule imaging in cells with near infra-red dyes[J]. PloS one, 2012, 7(4): e36265.
- [2] Kishimoto S, Bernardo M, Saito K, et al. Evaluation of oxygen dependence on in vitro and in vivo cytotoxicity of photoimmunotherapy using IR-700–antibody conjugates[J]. Free Radical Biology and Medicine, 2015, 85: 24-32.
- [3] Dou X, Nomoto T, Takemoto H, et al. Effect of multiple cyclic RGD peptides on tumor accumulation and intratumoral distribution of IRDye 700DX-conjugated polymers[J]. Scientific Reports, 2018, 8(1): 8126.
- [4] Nomoto T, Dou X. IRDye 700DX-conjugated polymers inducing photochemical internalization[J]. Photodiagnosis and Photodynamic Therapy, 2024, 46: 104166.

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