

Product Data Sheet

Product Name: PE-Cy7
Cat. No.: GC80383

Chemical Properties

Cas. No.

Formula

M.Wt

Solubility

Storage Store at 4°C, protect from 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

PE-Cy7

Background

PE-Cy7 is a tandem dye conjugate based on fluorescence resonance energy transfer (FRET). Composed of phycoerythrin (PE) as the donor and Cy7 as the acceptor fluorescent dye, PE-Cy7 is suitable for multicolor flow cytometry, laser scanning cytometry and immunofluorescence applications. PE-Cy7 can be conjugated to antibodies or used in the biotin-streptavidin system to label cell surface antigens in immunophenotyping and multiparametric cell analysis. PE-Cy7 undergoes photon-induced oxidative degradation, whose rate depends on light intensity and storage conditions, leading to reduced FRET efficiency and increased PE leakage. PE-Cy7 can be excited at wavelengths of 488 nm, 532 nm or ~760 nm, and its emission signal can be detected using 780/60

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nm, 810/90 nm or 785/50 nm bandpass filters, with a Stokes shift of 300 nm [1] [2] [3].

In Vitro, Operating Instructions (The following is a recommended procedure for reference only, and adjustments are required based on specific requirements)

1. Stock Solution Preparation

1.1 Solvents:

1.1.1 Storage buffer containing 0.016 M phosphate buffer (pH 7.4), 0.02% sodium azide and 0.2% bovine serum albumin.

1.1.2 Anhydrous DMSO.

1.2 Concentration Recommendations:

1.2.1 The concentration of bis-Cy7 stock solution is 10 mg/mL (10 mM).

1.2.2 Note: No stock solution concentration is specified for PE-Cy7 supplied as a conjugated antibody/streptavidin conjugate.

2. Working Solution Preparation

2.1 Diluent:

2.1.1 Dilute PE-Cy7-conjugated streptavidin or antibody at a ratio of 1:10 before use.

2.1.2 Note: PE-Cy7 supplied as a conjugated antibody/streptavidin conjugate requires no dilution and can be used directly.

2.2 Working Concentration: Optimize the concentration via titration to achieve saturated staining.

2.3 Note: Adjust the working solution concentration as needed; prepare and use it immediately.

3. Staining Procedure

3.1 Sample Types: Suitable for suspension cells (human peripheral blood mononuclear cell fractions, human peripheral blood leukocytes, peripheral blood mononuclear cells (PBMC))^{[1][2][3]}:

3.1.1 If whole blood leukocytes are used, process EDTA-anticoagulated blood samples.

3.1.2 Use at least 106 cells per stained sample.

3.1.3 Wash the cells twice before staining.

3.2 Incubation Conditions:

3.2.1 Direct staining: Incubate cells with PE-Cy7-conjugated antibody for 15 min at room temperature in the dark, or for 15 min at 0°C (ice bath); optimize conditions as needed.

3.2.2 Indirect staining: Aliquot blood samples and incubate with biotinylated primary antibody for 15 min at room temperature, then add PE-Cy7-conjugated streptavidin diluted at 1:10, and continue incubation for 15 min at room temperature.

3.3 Washing Steps:

3.3.1 After staining is completed, lyse red blood cells, then wash the samples with phosphate buffer.

3.3.2 Wash three times with staining medium (RPMI medium without biotin and flavins, supplemented with 4% fetal bovine serum and 0.02% sodium azide).

4. Control Setup

4.1 Set up unstained control, single-positive control, and Full-Minus-One control samples.

4.2 Positive controls are used to validate the experimental system; blank controls are used to eliminate reagent fluorescence interference.

5. Detection and Analysis

5.1 Instrument Types: Flow cytometer, laser scanning cytometer.

5.2 Excitation/Emission Wavelengths:

5.2.1 Excitation light: 532 nm laser, 488 nm argon ion laser.

5.2.2 Emission light: Detect via a 780/60 bandpass filter, detect via an 810/90 nm bandpass filter, or collect emission light >780 nm via a 50 nm bandpass filter with a central wavelength of 785 nm.

5.3 Result Analysis:

5.3.1 Changes in Fluorescence Intensity:

5.3.1.1 Degradation of PE-Cy7 leads to increased PE leakage (the ratio of donor dye emission to acceptor dye emission increases).

5.3.1.2 The PE-Cy7 fluorescence intensity of target-positive cell populations (e.g., CD3-positive lymphocytes) is significantly higher than that of target-negative cell populations.

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5.3.1.3 Before reaching the threshold, Cy7 fluorescence intensity increases with the rise of the molar ratio of Cy7 to PE; beyond the threshold, self-quenching causes a decrease in Cy7 fluorescence intensity; meanwhile, due to enhanced resonance energy transfer, PE fluorescence intensity decreases with the rise of the molar ratio of Cy7 to PE. 5.3.2 Fluorescence Localization: Targets to cell surface antigens via conjugated antibodies. 5.3.3 Other Parameters: 5.3.3.1 The resolution parameter of PE-Cy7 for distinguishing positive and negative cell populations is comparable to that of FITC and PE-Cy5. 5.3.3.2 Significant fluorescence spillover occurs into PE and PE-Cy5 channels; minimal spillover occurs into APC and APC-Cy7 channels. 5.3.3.3 Emits far-red fluorescence. 5.3.3.4 Instrument Settings (for laser scanning cytometer): Set the photomultiplier tube gain to 255, the offset to 2075, and the photomultiplier tube voltage to 55% of the maximum voltage.

References:

- [1]. Hulspas R, et al. Flow cytometry and the stability of phycoerythrin-tandem dye conjugates. Cytometry. Part A: the journal of the International Society for Analytical Cytology. 2009 Nov;75(11):966-72.
- [2]. Gerstner AO, et al. Near-infrared dyes for six-color immunophenotyping by laser scanning cytometry. Cytometry. 2002 Jul 01;48(3):115-23.
- [3]. Roederer M, et al. Cy7PE and Cy7APC: bright new probes for immunofluorescence. Cytometry. 1996 Jul 01;24(3):191-7.

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