RPE Tandem

Cat #: B-CHM311 / B-CHM312 / B-CHM314 / B-CHM315

Size: 1 mg / 10 mg

Storage: Store at 4°C protected from light.

Product specifications

Catalog No.	Name	Size	Ex/Em(nm)
B-CHM311	RPE-Cy7 Tandem	1mg	450-500nm/785nm
B-CHM312	Preactivated RPE-Cy5	10mg	450-500nm/667nm
B-CHM314	Preactivated RPE-Cy5.5	1mg	450-500nm/694nm
B-CHM315	Preactivated RPE-Cy7	1mg	450-500nm/785nm

Product Introduction

PE-Cy5 is a tandem dye that belongs to the phycobiliprotein conjugate family. In this type of tandem dye, the excitation energy can be transferred from PE to the Cy5, with the maximum emission wavelength being that of the Cy5.

PE-Cy5 has minimal spectral overlap with FITC and PE, resulting in low fluorescence interference. Therefore, it is often used in combination with FITC and PE in experiments. It is important to note that PE-Cy5 is not suitable for pairing with APC as they have a significant spectral overlap.

In contrast to PE-Cy5, PE-Cy5.5 has minimal fluorescence interference with FITC, PE, and APC, making it compatible for use in combination with these dyes. Additionally, PE-Cy5.5 exhibits higher fluorescence intensity compared to PE-Cy5.

PE-Cy7 has no spectral overlap with FITC and minimal overlap with APC, allowing it to be used in combination with FITC, PE, and APC. However, it is crucial to note that PE-Cy7 is highly susceptible to photobleaching and requires a strictly light-protected environment during experiments.

Product Properties

Form: Solution

Purity: Amax/A280 >5.0

Shipping and Storage

Storage conditions: Store at 4°C protect from light, do not freeze.

Stability: Stable for at least 6 months under proper storage conditions.

Instructions for Use

1. Antibody Modification

1) Dissolve the antibody modification reagent (DTT/TCEP) in ddH2O to prepare a 2 mg/ml antibody modification buffer.
2) Adjust the concentration of the antibody to be labeled (purity > 90%) to approximately 5-10 mg/ml using PBS buffer. Add 10 µl of the antibody modification buffer to per mg of antibody and gently mix. Stir the mixture at room temperature for 60-90 minutes.
3) After the reaction is complete, transfer the mixture to a centrifugal filter tube and add MES buffer. Centrifuge the filter multiple times to remove excess antibody modification buffer. The collected solution in the filter is the modified antibody, which should be adjusted to a concentration of 1-5 mg/ml.

2. PE Tandem Activation

1) Dissolve the PE tandem in 0.1M PBS (pH 7.4, 5mM EDTA) at a concentration of 5-10mg/ml.
2) Dissolve SMCC in anhydrous DMSO to prepare a 10mg/ml stock solution.
3) Add 5µl of SMCC per mg of PE tandem dye (n(PE tandem):n(SMCC) = 1:80). Seal the reaction mixture with aluminum foil and rotate at room temperature for 1 hour to allow the amino groups on the PE tandem to react with the succinimidyl ester, forming derivatized PE tandem.
4) After the reaction is complete, transfer the reaction mixture to an ultrafiltration centrifuge tube and centrifuge at 4°C, 12000 g for 5 minutes to remove the filtrate. Add 500µl of MES buffer, mix, and centrifuge. Repeat this step 5 times.
5) Collect the activated PE tandem solution.

3. Conjugation of Activated PE Tandem with Antibodies

1) Adjust the concentration of activated PE tandem to 5 mg/ml using MES buffer. To obtain the accurate weight of activated PE tandem, we recommend using the extinction coefficient of activated PE tandem for measurement ([PE tandem] = 0.12245 × A565, where [PE tandem] is the concentration in mg/ml and A565 is the absorbance at 565 nm, preferably within the range of 0.3-0.8).
2) Mix the modified antibody with activated PE tandem at a mass ratio of 1:3 (3 mg of activated PE tandem per mg of modified antibody). Perform the reaction at room temperature, protected from light, for 2 hours.
3) Dissolve NEM in anhydrous dimethyl sulfoxide (DMSO) to prepare a 12.5 mg/ml solution (0.1 M). Add 5 µl of NEM solution per mg of antibody to the reaction mixture from step 2) to block any remaining active groups.

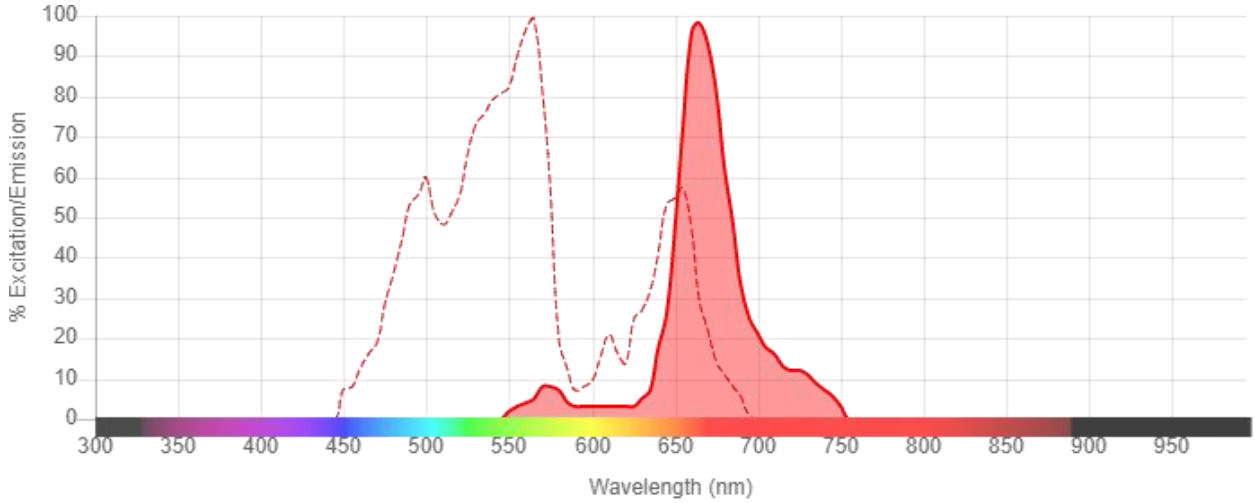
4) Transfer the solution from step 3) to the centrifugal filter tube and remove the blocking buffer through repeated centrifugation.

5) Divide the labeled antibody into aliquots, add appropriate preservatives, and store at -20°C for future use.

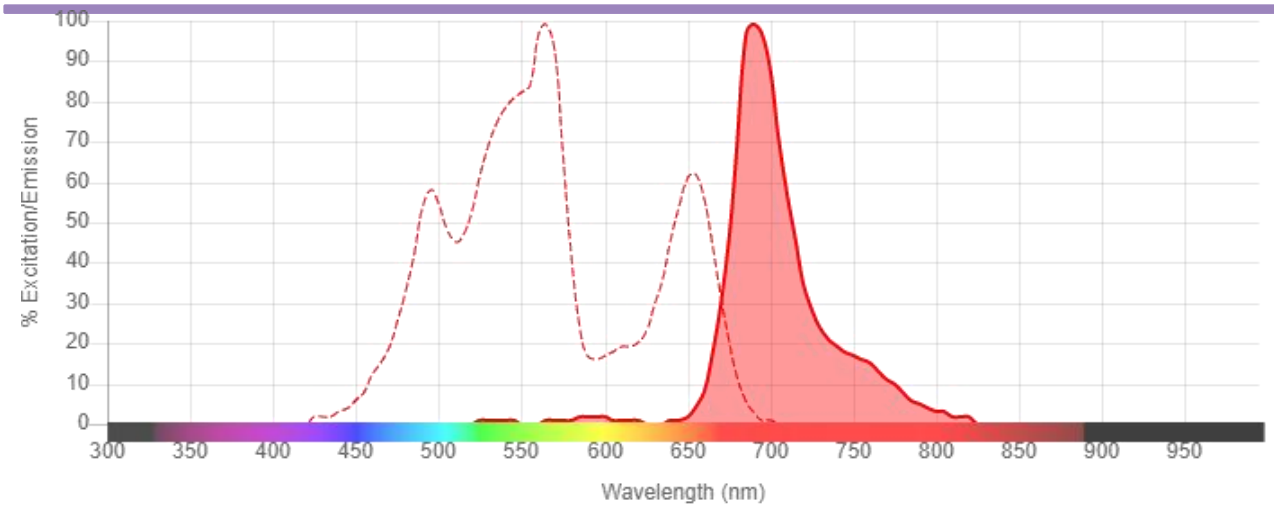
Precautions:

1. Activated PE tandem should be stored in the dark at cold. During the labeling process, it should be protected from light as much as possible.
2. The blocking reagent and modification reagent should be prepared fresh and used immediately. They should not be stored for a long time.
3. The labeled antibody should have high specificity and a purity of no less than 90%. Monoclonal antibodies are preferred, and the solution should not contain free amines. It is best to use PBS. Prior to labeling, the antibody should be depleted of NaN3 and BSA. Operations such as dialysis, concentration, and concentration measurement can cause loss of antibody. Therefore, the appropriate amount of antibody for labeling should be determined based on specific circumstances.
4. Due to the susceptibility of the introduced groups in the modified antibody to reoxidation, the modified antibody should be conjugated with activated PE tandem as soon as possible after modification.

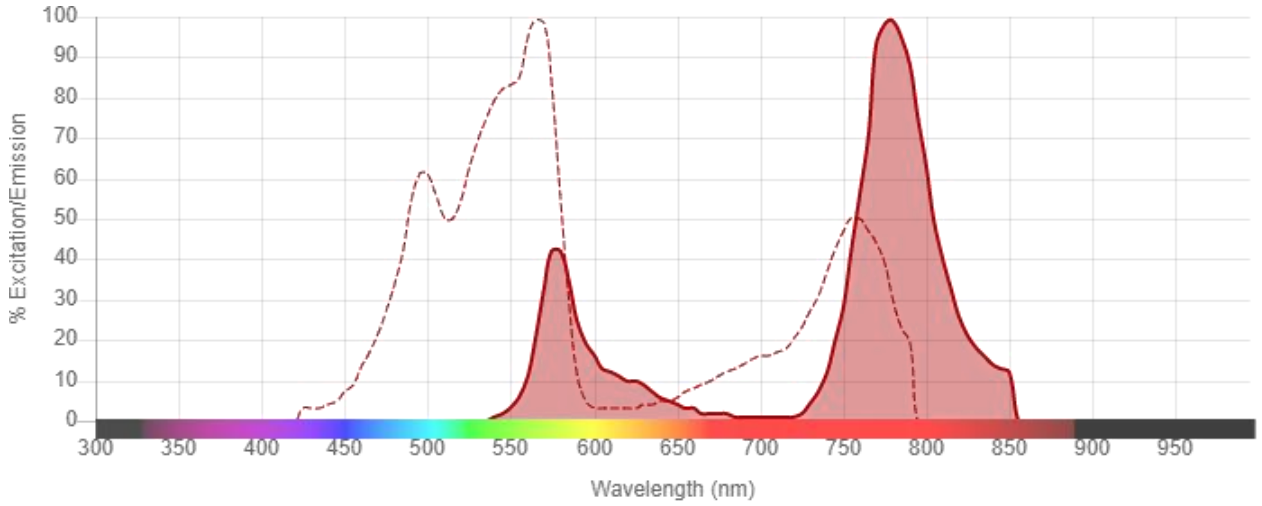
PE-Cy5:



PE-Cy5.5:



PE-Cy7:



Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.