Preactivated PerCP-Cy5.5

Cat #: B-CHM313

Size: 10 mg

Storage: Store at 4°C protected from light.

Product Introduction

Peridinin-Chlorophyll-Protein (PerCP) is a complex with a molecular weight of 35.5 kDa, isolated from dinoflagellates (Dinophyceae sp.). It consists of multiple peridinin-chlorophyll-protein complexes. PerCP has a broad excitation spectrum with a peak at 482 nm and a significant Stokes shift, with the highest emission peak at 677 nm. PerCP and its conjugates are commonly used in cell surface labeling techniques such as flow cytometry.

PerCP-Cy5.5 is a stable tandem dye that is excited by a 488 nm laser. It allows staining of low-abundance targets. In multicolor flow cytometry, the Cyanine5.5 signal can be reliably detected. When detecting PerCP-Cy5.5 and APC together in dual-laser instruments, compensation adjustments are required, but fewer adjustments are needed compared to PerCP.

PerCP conjugates easily with antibodies and other proteins without the need for chemical cross-linkers. These highly purified PerCP proteins retain their spectral characteristics after conjugation. Our activated PerCP proteins are treated with succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), which reacts with lysine residues, allowing the maleimide group to react with free thiol groups on the conjugate partner protein. They are ready to use and can be directly conjugated with thiol-containing targets without further processing.

Product Properties

Form: Solution

Spectral properties: Ex / Em = 488 nm / 702 nm

Purity: A482/A280 >4.2

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.

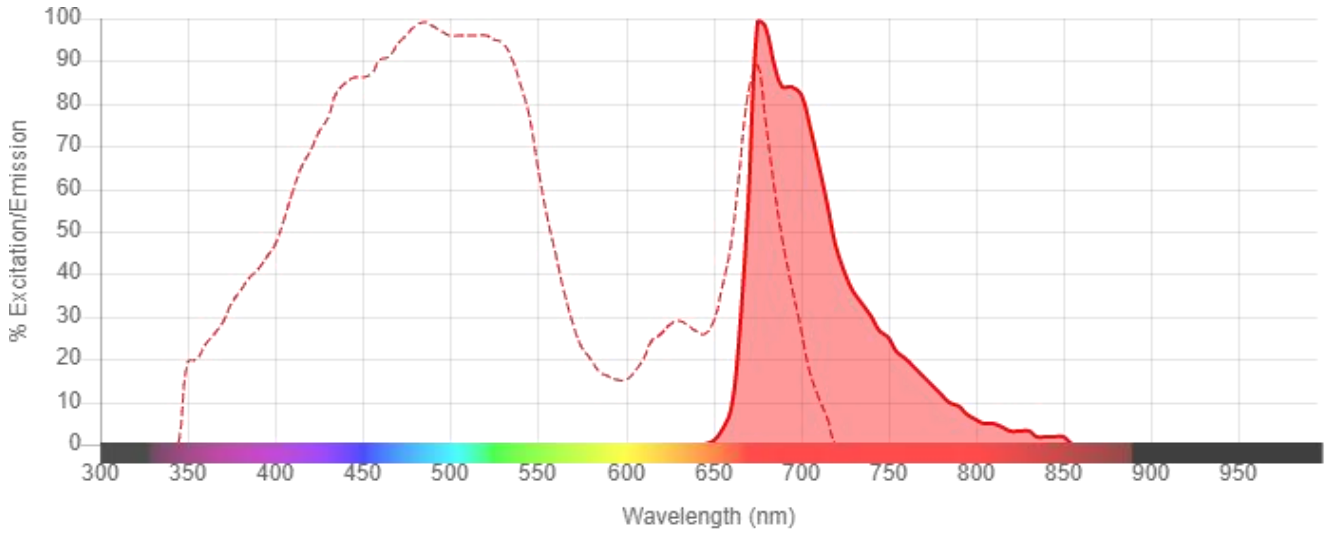
2. Conjugation of Activated PerCP-Cy5.5 with Antibodies

1) Adjust the concentration of activated PerCP-Cy5.5 tandem to 5 mg/ml using MES buffer. To obtain the accurate weight of activated PerCP tandem, we recommend using the extinction coefficient of activated PerCP-Cy5.5 for measurement ([PerCP-Cy5.5 tandem] = 0.086 × A482, where [PerCP-Cy5.5 tandem] is the concentration in mg/ml and A482 is the absorbance at 482 nm, preferably within the range of 0.3-0.8).
2) Mix the modified antibody with activated PerCP-Cy5.5 tandem at a mass ratio of 2.25:1 (0.45 mg of activated PerCP-Cy5.5 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 PerCP-Cy5.5 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 PerCP-Cy5.5 tandem as soon as possible after modification.

PerCP-Cy5.5:



Note:

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