Preactivated PerCP

Cat #: B-CHM307

Size: 1mg/5mg/10mg/50mg/100mg/

Storage: Store at 2-8°C protected from light, do not freeze.

Product Introduction

PerCP (Peridinin-chlorophyll-protein complex) is isolated from Dinophyceae sp. It has an extremely high extinction coefficient, a high quantum efficiency and a large Stokes shift. It is well excited with the Argon laser at 482 nm with its maximum emission peak at 677nm. PerCP protein is commonly used for fluorescent immunolabeling, particularly in applications involving fluorescent-activated cell sorting (FACS). Its cyanine tandem conjugates (such as PerCP-Cy5.5 developed by BD) can be excited with a standard 488 nm laser and emits in the far red at a longer wavelength for multicolor flow cytometric analysis of cells.

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

Product Properties

Form: Solution

Molecular weight: 35500 Dalton

Spectral properties: Ex / Em = 488 / 675±5 nm

Purity: A_{482}/A_{280} >4.2, A_{620}/A_{482}<0.005

Quantitative method: In order to obtain the precise weight of PerCP, we recommend using the extinction coefficient of PerCP as a reference (i.e. [PerCP]=0.086 × A_{482}, where [PerCP] is the concentration in mg/ml, and A_{482} is the absorbance value at a wavelength of 482nm, and the A_{482} should within a range of 0.3 to 0.8.

Shipping and Storage

Storage conditions: Store at 2-8°C protected from light, do not freeze.

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

Instructions for Use

1. Antibody Modification

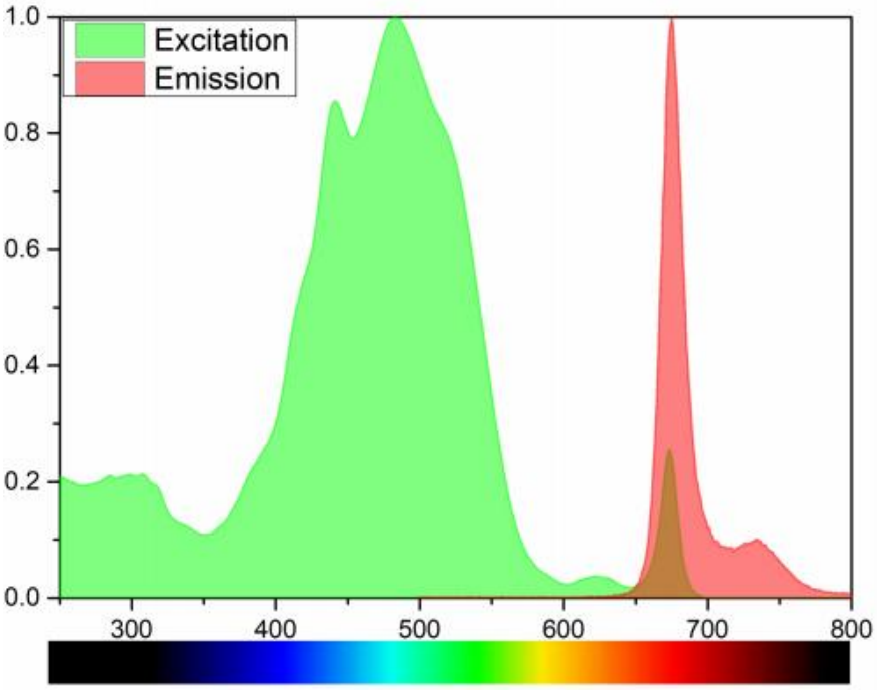
1) Dissolve the antibody modification reagent (DTT/TCEP) in ddH2O to prepare a 2 mg/ml antibody modification buffer.
2) Adjust the concentration of the antibody to be labeled (purity > 90%) to approximately 5-10 mg/ml using PBS buffer. Add 10 µl of the antibody modification buffer to per mg of antibody 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 Preactivated PerCP with Antibodies

1) Adjust the concentration of Preactivated PerCP to 5 mg/ml using MES buffer. To obtain the accurate weight of PerCP, we recommend using the extinction coefficient of PerCP for measurement ([PerCP] = 0.086 × A_{482}, where [PerCP] is the concentration in mg/ml and A_{482} is the absorbance at 482 nm, preferably within the range of 0.3-0.8).
2) Mix the modified antibody with Preactivated PerCP at a mass ratio of 1:1.2 (1.2 mg of Preactivated PerCP 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. Preactivated PerCP 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 as soon as possible after modification.


Excitation
Emission
1.0
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0.2
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300
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800