Preactivated APC [Allophycocyanin]

Cat #: B-CHM306

Size: 1 mg

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

Product Introduction

Allophycocyanin (APC) is a phycobiliprotein isolated from Spirulina sp., a blue-green alga. Like other phycobiliproteins, APC is fluorescent, with an extremely high absorptivity and high quantum efficiency. It is a protein which can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics. Allophycocyanin is the least stable among the major phycobiliproteins, susceptible to dissociation at low concentrations including concentrations at which some assays are performed.

Activated allophycocyanin is easily conjugated with antibodies and other proteins without the need for chemical cross-linkers. These highly purified allophycocyanin retain their spectral characteristics after conjugation. The activated allophycocyanin 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

Spectral properties: Ex / Em = 652 / 662 nm

Purity: Amax/A280 >4.5

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

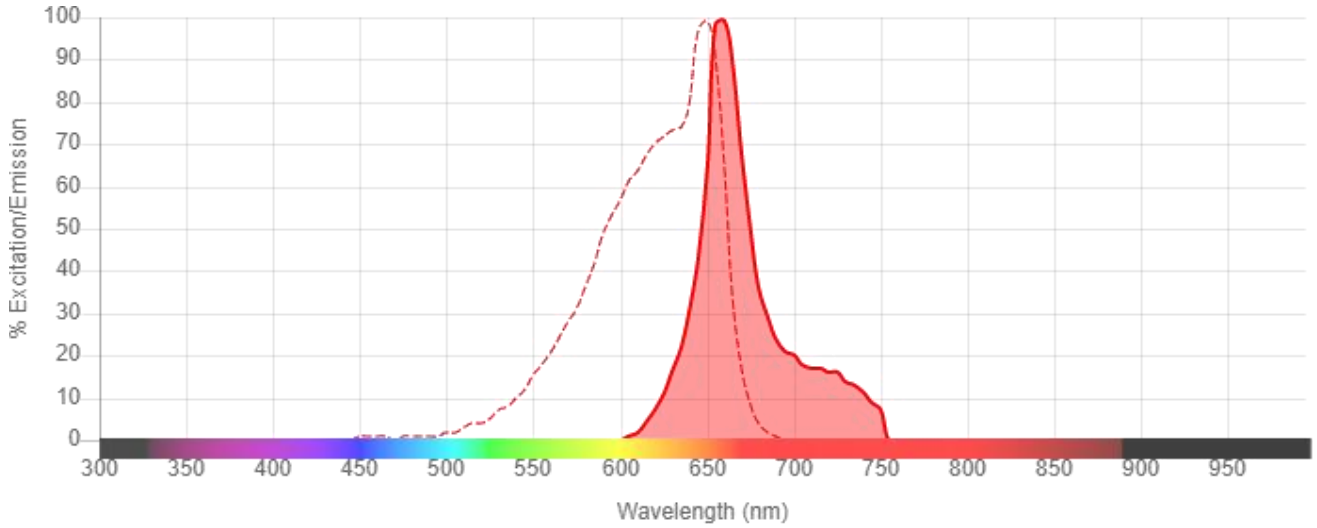
1) Dissolve the antibody modification reagent (DTT/TCEP) in ddH2O to prepare a 2 mg/ml antibody modification buffer.
2) Adjust the concentration of the antibody to be labeled (purity > 90%) to approximately 5-10 mg/ml using PBS buffer. Add 10 µl of the antibody modification buffer to per mg of antibody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

1) Adjust the concentration of Activated Allophycocyanin to 5 mg/ml using MES buffer. To obtain the accurate weight of APC, we recommend using the extinction coefficient of APC for measurement ([APC] = 0.14999 × A651, where [APC] is the concentration in mg/ml and A651 is the absorbance at 651 nm, preferably within the range of 0.3-0.8).
2) Mix the modified antibody with Activated Allophycocyanin at a mass ratio of 1:1.3 (1.3 mg of Activated Allophycocyanin 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 Allophycocyanin 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 APC as soon as possible after modification.



Note:

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