APC Tandem

Cat #: B-CHM310 / B-CHM316 / B-CHM317

Size: 1 mg

Storage: Store at 4°C protected from light.

Product specifications

Catalog No.	Name	Ex/Em(nm)
B-CHM310	APC-Cy7 Tandem	651nm/780nm
B-CHM316	Preactivated APC-AF700	651nm/710nm
B-CHM317	Preactivated APC-AF750	651nm/780nm

Product Introduction

Allophycocyanin (APC) is a phycobiliprotein isolated from spirulina, a type of blue-green algae. Like other phycobiliproteins, APC exhibits fluorescence and has a high extinction coefficient and quantum yield. It can be easily conjugated to antibodies and other proteins using conventional protein crosslinking techniques without altering its spectral properties. Its strong absorption at 633nm and 647nm makes it and its tandems one of the preferred fluorochromes used in conjunction with red lasers in flow cytometry applications.

APC-AF700 and APC-Alexa Fluor 700 tandem dyes have the same structure. Their main absorption peak is at 651nm, and the emission peak is around 710nm.

APC-AF750 tandem is an excellent alternative to APC-Cy7, offering higher FRET efficiency and signal. Its main absorption peak is at 651nm, and the emission peak is around 780nm.

Product Properties

Form: Solution

Laser: Laser633

Shipping and Storage

Storage conditions: Store at 4°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. APC Tandem Activation

1) Dissolve the APC 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 APC tandem dye (n(APC 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 APC tandem to react with the succinimidyl ester, forming derivatized APC 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 APC tandem solution.

3. Conjugation of Activated APC Tandem with Antibodies

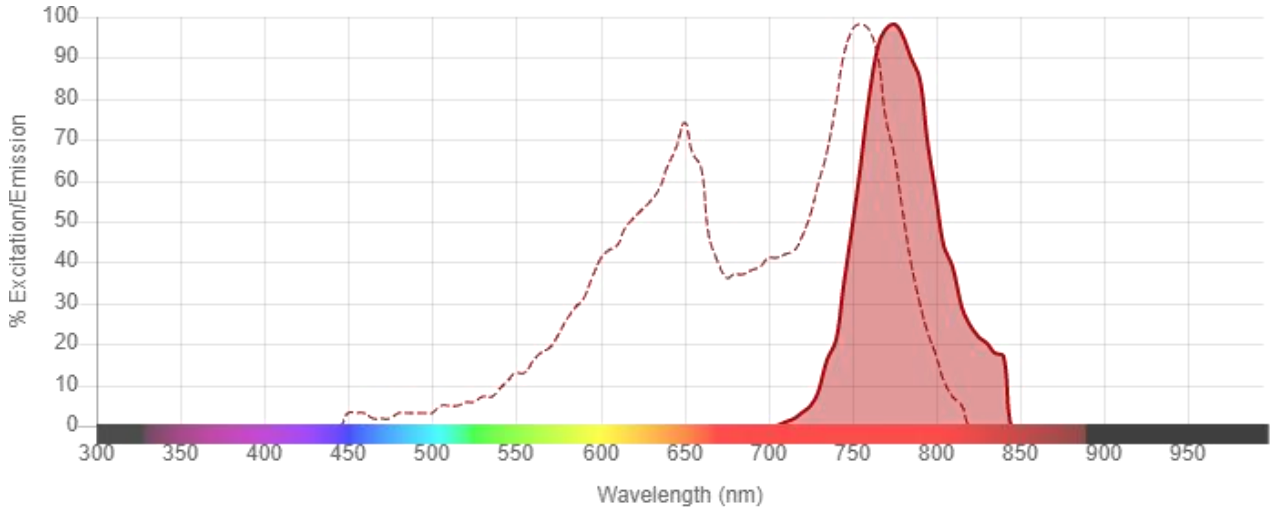
1) Adjust the concentration of activated APC tandem to 5 mg/ml using MES buffer. To obtain the accurate weight of activated APC tandem, we recommend using the extinction coefficient of activated APC tandem for measurement ([APC tandem] = 0.14999 × A651, where [APC tandem] is the concentration in mg/ml and A651 is the absorbance at 651 nm, preferably within the range of 0.3-0.8).

2) Mix the modified antibody with activated APC tandem at a mass ratio of 1:1.3 (1.3 mg of activated APC 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.

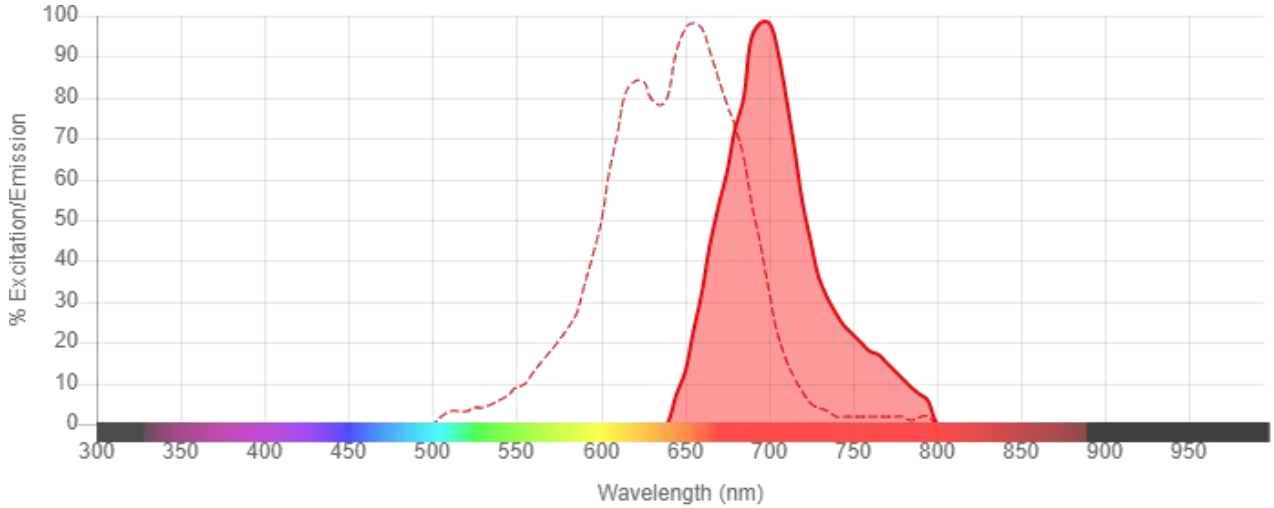
Precautions:

1. Activated APC 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 APC tandem as soon as possible after modification.

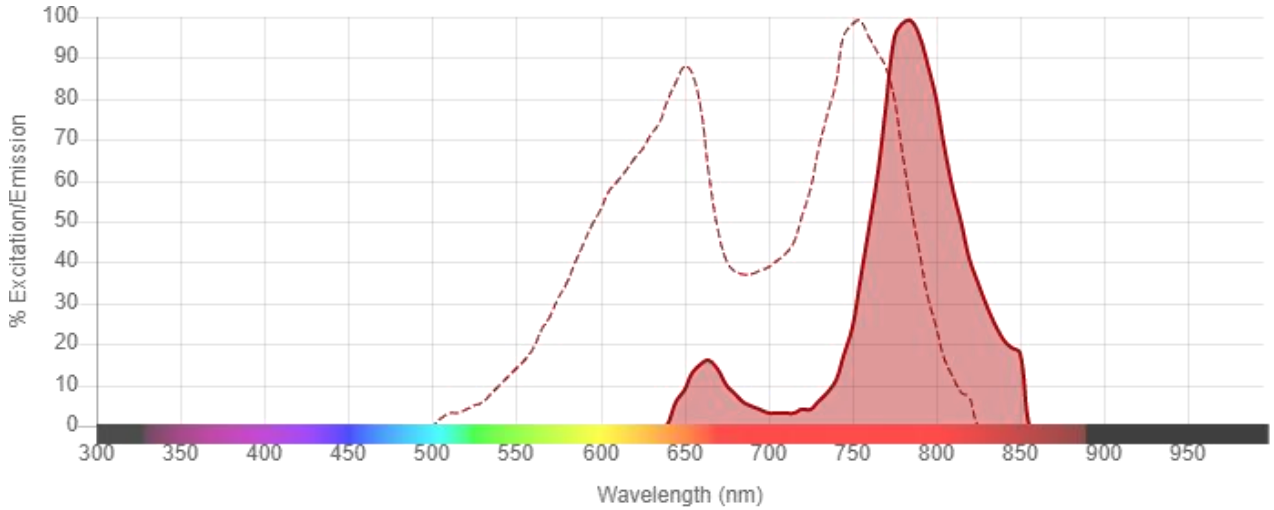
APC-Cy7:



APC-AF700:



APC-AF750:



Note:

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