Activated R-Phycoerythrin

Cat #: B-CHM305

Size: 1 mg

Storage: Store at 4°C, protected from light.

Product Introduction

Phycoerythrin is isolated and purified from red algae, capable of emitting strong fluorescence, excellent absorbance, and high quantum yield. It has a wide excitation and emission range in the visible spectral region. By using conventional labeling methods, it can be easily combined with biotin, avidin, and various monoclonal antibodies to form fluorescent probes, which are used for antibody fluorescence labeling such as fluorescence microscopy, fluorescence immunoassay, dual or multicolor fluorescence analysis, cancer cell surface antigen detection, flow cytometry fluorescence measurement, and diagnostic and bioengineering technologies such as in vivo imaging applications.

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

Spectral properties: Ex / Em = 450-500 nm / 575 ± 5 nm

Purity: Amax/A280 >5.0

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. Conjugation of Activated R-Phycoerythrin with Antibodies

1) Adjust the concentration of Activated R-Phycoerythrin to 5 mg/ml using MES buffer. To obtain the accurate weight of R-PE, we recommend using the extinction coefficient of R-PE for measurement ([R-PE] = 0.12245 × A565, where [R-PE] 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 R-Phycoerythrin at a mass ratio of 1:3 (3 mg of Activated R-Phycoerythrin 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 R-Phycoerythrin 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 R-PE as soon as possible after modification.