

ReadiUse™ Preactivated APC Maleimide [Activated Allophycocyanin]

Catalog number: 2567, 2568
Unit size: 1 mg, 5 mg

Component	Storage	Amount (Cat No. 2567)	Amount (Cat No. 2568)
ReadiUse™ Preactivated APC Maleimide [Activated Allophycocyanin]	Refrigerated (2-8 °C), Minimize light exposure	1 mg	5 mg

OVERVIEW

ReadiUse™ Preactivated APC Maleimide is prepared by reacting highly purified allophycocyanin (APC) with SMCC. The NHS group of SMCC reacts with the lysine groups of APC, leaving maleimide groups available to react with free sulfhydryl groups on proteins to be conjugated. ReadiUse™ Preactivated APC Maleimide is highly purified to completely remove the unreacted SMCC, and lyophilized to powder. It is ready to use and will conjugate without further preparation upon mixing with sulfhydryl-containing target molecules such as reduced IgGs. The activated allophycocyanin can be easily conjugated to antibodies and other proteins without the use of added chemical crosslinking agents. These highly purified phycobiliproteins maintain their spectral characteristics of APC.

SAMPLE EXPERIMENTAL PROTOCOL

Reduction of Antibody

1. Prepare a fresh solution of 1.0 M DTT (15.4 mg/100 µL) in distilled water. Antibody reduction can be carried out in different buffers, such as MES, phosphate, or TRIS (pH 6 to 8). For best results, antibody solutions should be >2 mg/mL. If less than 2 mg/mL, the antibody should be concentrated.
2. Add 2 µL of 1.0 M DTT stock per 100 µL of antibody solution, and mix well. Let the antibody solution stand at room temperature for 30 minutes without additional mixing to minimize the reoxidation of cysteines.
3. Purify the reduced antibody using a desalting column (Cat No. 60500) pre-equilibrated with 50 mM MES buffer (pH 6.0-6.5) and 2 mM EDTA.
4. Measure the antibody concentration using a Nanodrop. (Con. (mg/mL) = A280nm/1.4).

Note Reduced antibodies are typically unstable. It is best to run the conjugation reaction immediately following purification.

Conjugate with ReadiUse™ Preactivated APC Maleimide

1. Reconstitute ReadiUse™ Preactivated APC Maleimide in ddH₂O to make a 10 mg/mL solution. For Cat No. 2567 (1 mg) reconstitute in 100 µL of ddH₂O. If using Cat No. 2568 (5 mg), reconstitute in 500 µL of ddH₂O. Reconstituted ReadiUse™ Preactivated APC Maleimide solutions are stable at 4 °C for up to one month, protected from light.
2. Add the reduced antibody directly to the solution of ReadiUse™ Preactivated APC at a ratio of 130 µg APC/100 µg of reduced antibody.
3. Rotate the mixture for 60 to 120 minutes at room temperature.
4. Block any free sulfhydryl groups on the antibody. Prepare a fresh solution of 10 mg/mL N-Ethylmaleimide (NEM) in DMSO and add 3.4 µL per mg of antibody. Rotate for 20 minutes at room temperature.

Conjugate Purification

1. Purify the antibody/APC conjugate solution using size exclusion chromatography.

Note Typically the antibody/APC conjugate can be stored at 2-8 °C, protected from light, for up to 6 months. The best storage conditions for any particular conjugate must be determined by experimentation and depends on the stability of the antibody.

DISCLAIMER

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