

ReadiUse™ Preactivated PE Maleimide [Activated R-Phycocerythrin]

 Catalog number: 2565, 2566
 Unit size: 1 mg, 5 mg

Component	Storage	Amount (Cat No. 2565)	Amount (Cat No. 2566)
ReadiUse™ Preactivated PE Maleimide [Activated R-Phycocerythrin]	Desiccated, Refrigerated (2-8 °C), Minimize light exposure	1 mg	5 mg

OVERVIEW

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

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

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

SAMPLE EXPERIMENTAL PROTOCOL

Reduction of IgG

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 range 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 PE Maleimide

1. Reconstitute ReadiUse™ Preactivated PE Maleimide in ddH₂O to make a 10 mg/mL solution. For Cat No. 2565 (1 mg) reconstitute in 100 µL of ddH₂O. If using Cat No. 2566 (5 mg), reconstitute in 500 µL of ddH₂O. Reconstituted ReadiUse™ Preactivated PE Maleimide solutions are stable at 4 °C for up to one week, protected from light.
2. Add the reduced antibody directly to the solution of ReadiUse™ Preactivated PE at a ratio of 250-300 µg PE/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/PE conjugate solution using size exclusion chromatography. **Note:** Typically the antibody/PE conjugate can be