

ReadiUse™ Preactivated PE-Cy5.5 MaleimideCatalog number: 2718
Unit size: 1 mg

Component	Storage	Amount
ReadiUse™ Preactivated PE-Cy5.5 Maleimide	Refrigerated (2-8 °C), Minimize light exposure	1 mg

OVERVIEW

PE-Cy5.5 is a popular tandem color used in flow cytometry. Its primary absorption peak is at 565 nm with emission peak at ~700 nm. AAT Bioquest offers this preactivated PE-Cy5.5 maleimide to facilitate the PE-Cy5.5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. It is prepared from the commonly used crosslinker SMCC. Our preactivated PE-Cy5.5 maleimide is ready to conjugate the reduced antibodies and other thiol-containing biomolecules.

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 solutions should be at 2 mg/mL or higher for best results. The reduction can be carried out in different buffers for example: MES, phosphate, and TRIS buffers (pH range 6 to 8). The antibody should be concentrated if less than 2 mg/mL.
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 temp for 30 minutes without additional mixing (to minimize reoxidation of cysteines to cystines).
3. Purify the reduced antibody over a desalting column pre-equilibrated with 50 mM MES Buffer (pH=6.0-6.5) with 2 mM EDTA. (Desalting column: <https://www.aatbio.com/products/readiuse-bio-gel-p-6-spin-column?unit=60500>)
4. Measure the Antibody concentration with Nanodrop. (Con. (mg/ml)= A280nm/1.4) **Note:** The reduced antibody is not stable; the conjugation reaction needs to be carried out the conjugation as soon as possible after purification.

Conjugate with ReadiUse™ Preactivated PE-Cy5.5 Maleimide

1. Reconstitute ReadiUse™ Preactivated PE-Cy5.5 Maleimide in 100 µL ddH₂O to 10 mg/mL. Note: Reconstituted ReadiUse™ Preactivated PE-Cy5.5 Maleimide are stable at 4 °C for one week, please kept it from light.
2. Add reduced antibody to pre-activated PE-Cy5.5 directly to at the ratio of 250-300 µg PE-Cy5.5 /100 µg reduced antibody.
3. Rotate the mixture for 60-120 mins at room temperature.
4. After 60 minutes, block the free sulfhydryls on the antibody.
5. Prepare a fresh solution of 10 mg/mL NEM in DMSO; add 3.4 µL per mg of antibody and rotate for 20 minutes at room temperature.

Purification

1. Antibody/PE-Cy5.5 conjugate could be further purified through size exclusion chromatography to get best performance. **Note:** The Antibody/PE-Cy5.5 conjugate solution is recommended to be stored at 2-8 °C and kept from light.