

## ReadiUse™ Preactivated APC-Cy5.5 Maleimide

Catalog number: 2721  
Unit size: 1 mg

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

### OVERVIEW

APC-Cy5.5 is a popular tandem color used in flow cytometry. Its primary absorption peak is at 651 nm with emission peak at ~700 nm. AAT Bioquest offers this preactivated APC-Cy5.5 to facilitate the APC-Cy5.5 tandem conjugations to reduced antibodies and other biomolecules that contain a thiol group. Our preactivated APC-Cy5.5 maleimide is prepared from the commonly used crosslinker SMCC, and ready to conjugate. Allophycocyanin (APC) is a phycobiliprotein isolated from *Spirulina* sp., a blue-green alga. Like other phycobiliproteins, APC is fluorescent, with an extremely high absorptivity and a high quantum efficiency. It is a protein which can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics.

Antibody/APC-Cy5.5 conjugate solution is recommended to be stored at 2~8 °C and kept from light.

### DISCLAIMER

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### 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 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 APC-Cy5.5 Maleimide

1. Reconstitute ReadiUse™ Preactivated APC-Cy5.5 Maleimide in 100 µL ddH<sub>2</sub>O to 10 mg/mL. Note: Reconstituted ReadiUse™ Preactivated APC-Cy5.5 Maleimide are stable at 4 °C for one week, please keep it from light.
2. Add reduced antibody to pre-activated APC-Cy5.5 directly to at the ratio of 130 µg APC-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/APC-Cy5.5 conjugate could be further purified through size exclusion chromatography to get best performance. **Note:** The