

## ReadiUse™ Preactivated APC-Cy5.5 Tandem

 Catalog number: 2586  
 Unit size: 1 mg

Component	Storage	Amount
A: ReadiUse™ Preactivated APC-Cy5.5 Tandem	Refrigerated (2-8 °C), Minimize light exposure	1 vial (1 mg)
B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µg)
C: Spin Desalting Column		Not Included

### OVERVIEW

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. APC-Cy5.5 is a popular 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 antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated APC-Cy5.5 tandem is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated APC-Cy5.5 tandem is conjugated to a protein via its amino group that is abundant in proteins while SMCC chemistry targets the thiol group that has to be regenerated by the reduction of antibodies.

### SAMPLE EXPERIMENTAL PROTOCOL

#### Preparation of pre-activated antibody with Buccutite™ MTA

1. Reconstitute Buccutite™ MTA in DMSO at ~10 mg/mL.
 

**Note** Please store unused Buccutite™ MTA at -20 °C and could be used up to two freeze and thaw cycles.
2. Prepare target antibody (Ab) in pH = 8.5 - 9.0 buffer at concentration above 1 mg/mL.
3. Add Buccutite™ MTA to Ab solution at the ratio of 8 - 10 µg Buccutite™ MTA/100 µg Ab.
4. Mix well and react at room temperature for 60 minutes, rotating during the reaction.
5. Purify the reaction mixture with desalting column to remove unreacted Buccutite™ MTA and exchange buffer to PBS or buffer of your choice.
6. Collect the Buccutite™ MTA-activated Ab, and estimate the concentration by 70% yield of the original starting amount.

#### Conjugation with pre-activated APC-Cy5.5

1. Reconstitute pre-activated APC-Cy5.5 in 100 µL ddH<sub>2</sub>O to 10 mg/mL.
 

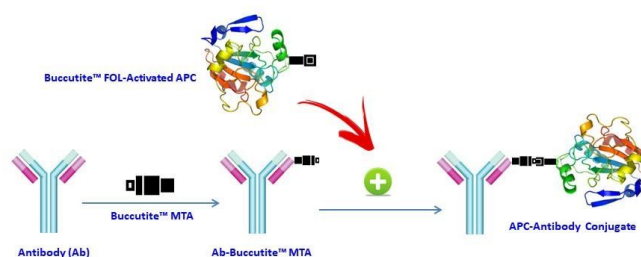
**Note** Reconstituted pre-activated APC-Cy5.5 could be stored at 4 °C for one month, kept from light.
2. Add APC-Cy5.5 directly to MTA-activated target Ab solution at the ratio of 130 µg APC-Cy5.5/100 µg MTA-activated Ab.
3. Rotate the mixture for 60 minutes at room temperature.
4. The Ab/APC-Cy5.5 conjugates are now ready to use.

**Note** The antibody conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin)

and 0.02% - 0.05% sodium azide. The Ab/APC-Cy5.5 conjugates solution could be stored at 4 °C for up to two months, and kept from light.

5. (Optional) Ab/APC-Cy5.5 conjugates could be further purified through size exclusion chromatography to get best performance.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** Our preactivated APC-Cy5.5 was premodified with our Buccutite™ FOL (provided). Your antibody (or other proteins) is modified with our Buccutite™ MTA (provided as free sample) to give MTA-modified protein (such as antibody). The MTA-modified protein readily reacts with FOL-modified APC-Cy5.5 (provided) to give the desired APC-Cy5.5-antibody conjugate in much higher yield than the SMCC chemistry. In addition our preactivated APC-Cy5.5 reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

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