

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 ddH₂O 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

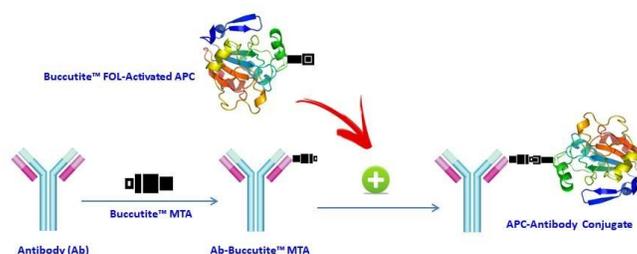


Figure 1. Our preactivated APC-Cy5.5 was premodified with our Buccutite™ FOL (provided). Your antibody (or other proteins) is modified with our Buccutite™ MTA (provided as free sample) to give MTA-modified protein (such as antibody). The MTA-modified protein readily reacts with FOL-modified APC-Cy5.5 (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.

DISCLAIMER

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