

## ReadiUse™ Preactivated APC

Catalog number: 2561

Unit size: 1 mg

Component	Storage	Amount
ReadiUse™ Preactivated APC	Refrigerate (2-4 °C), Minimize light exposure	1 mg

### 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. AAT Bioquest offers this preactivated APC to facilitate the APC conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated APC is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated APC 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.

### AT A GLANCE

#### Important

##### Conjugation principle

APC was premodified with our Buccutite™ FOL. Your antibody (or other proteins) is modified with our Buccutite™ MTA to give MTA-modified protein. The MTA-modified protein readily reacts with FOL-modified APC (provided) to give the desired APC-antibody conjugate.

### SAMPLE EXPERIMENTAL PROTOCOL

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

1. Reconstitute Buccutite™ MTA in DMSO at ~10 mg/mL.
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, APC-iFluors, or APC-iFluor tandems

1. Reconstitute pre-activated APC, APC, APC-iFluors, or APC-iFluor tandems in 100 µL ddH<sub>2</sub>O to 10mg/mL.
2. Add APC, APC-iFluors, or APC-iFluor tandems directly to MTA-activated target Ab solution at the ratio of 130 µg APC, APC-iFluors, or APC-iFluor tandems/100µg MTA-activated Ab.
3. Rotate the mixture for 60 minutes at room temperature.
4. The Ab/APC, APC-iFluors, or APC-iFluor tandems 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, APC-iFluors, or APC-iFluor tandems conjugates solution could be stored at 4°C for up to two months, and kept from light.

5. (Optional) Ab/APC, APC-iFluors, or APC-iFluor tandems conjugates could be further purified through size exclusion chromatography to get best performance.

### EXAMPLE DATA ANALYSIS AND FIGURES

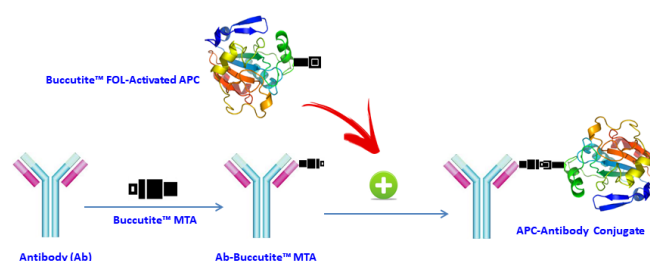


Figure 1.

Our preactivated APC 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 (provided) to give the desired APC-antibody conjugate in much higher yield than the SMCC chemistry. In addition our preactivated APC reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

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