

## ReadiUse™ Preactivated PE

Catalog number: 2560

Unit size: 1 mg

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

### OVERVIEW

R-Phycoerythrin (PE) is isolated from red algae. Its primary absorption peak is at 565 nm with secondary peaks at 496 and 545 nm. AAT Bioquest offers this preactivated PE to facilitate the PE conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PE is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PE 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** PE 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 PE (provided) to give the desired PE-antibody conjugate.

### SAMPLE EXPERIMENTAL PROTOCOL

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

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

**Note** Store unused MTA at -20°C; it can be used for up to two freeze and thaw cycles.

2. Prepare target antibody (Ab) in pH = 8.5 - 9.0 buffer at a concentration above 1 mg/mL.
3. Add the MTA to Ab solution at the ratio of 8 - 10 µg 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 a desalting column to remove any unreacted MTA. Exchange the buffer to PBS or another buffer of your choice.
6. Collect the MTA-activated Ab. Estimate the concentration by 70% yield of the original starting amount.

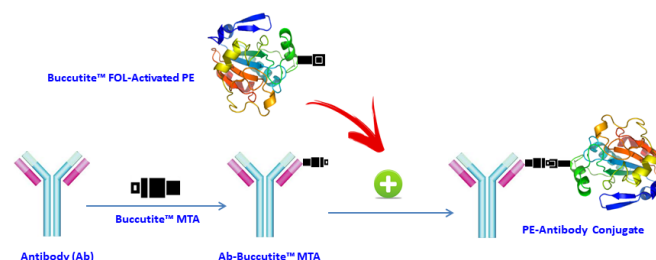
#### Conjugate with Pre-activated PE

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

**Note** Reconstituted pre-activated PE is not stable and can not be stored for more than one month.

2. Add pre-activated PE directly to MTA-activated target Ab solution at the ratio of 300 µg PE/100 µg MTA-activated Ab.
3. Rotate the mixture for 1 - 2 hours at room temperature.
4. The Ab/PE conjugates are now ready to use. 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/PE can be stored at 4°C for two months.
5. Optional: Ab/PE can be further purified through size exclusion chromatography to get better performance.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.**

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

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