![AAT Bioquest® logo]

ReadiUse™ Preactivated PE-Cy5.5 Tandem

Catalog number: 2581
Unit size: 1 mg

Component	Storage	Amount
A: ReadiUse™ Preactivated PE-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

PE-Cy5.5 is a popular color used in flow cytometry. Its primary absorption peak is at 565 nm with emission peak at~700 nm. The filter sets of 682/33 nm and 695/40 nm are recommended for this tandem color. AAT Bioquest offers this preactivated PE-Cy5.5 to facilitate the PE-Cy5.5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PE-Cy5.5 tandem is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PE-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.

AT A GLANCE

Important   PE-Cy5.5 Tandem 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-Cy5.5 Tandem (provided) to give the desired PE-Cy5.5 Tandem-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-Cy5.5 Tandem

1. Reconstitute pre-activated PE-Cy5.5 Tandem in 100 µL ddH$_2$O to 10 mg/mL.

    Note   Reconstituted pre-activated PE-Cy5.5 Tandem is not stable and can not be stored for more than one month.

2. Add pre-activated PE-Cy5.5 Tandem directly to MTA-activated target Ab solution at the ratio of 300 µg PE-Cy5.5 Tandem/100 µg MTA-activated Ab.

3. Rotate the mixture for 1 - 2 hours at room temperature.

4. The Ab/PE-Cy5.5 Tandem 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.

    Note   The Ab/PE-Cy5.5 Tandem can be stored at 4 °C for two months.

5. Optional: Ab/PE-Cy5.5 Tandem can be further purified through size exclusion chromatography to get better performance.
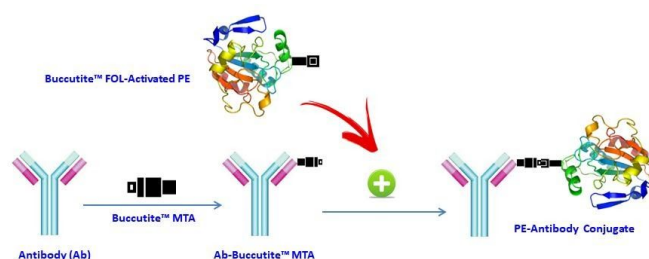
EXAMPLE DATA ANALYSIS AND FIGURES

Figure 1. Our preactivated PE-Cy5.5 Tandem 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-Cy5.5 Tandem (provided) to give the desired PE-Cy5.5 Tandem-antibody conjugate in much higher yield than the SMCC chemistry. In addition our preactivated PE-Cy5.5 Tandem reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

DISCLAIMER

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