

ReadiUse™ Preactivated PE-iFluor™ 660 Tandem

 Catalog number: 2579
 Unit size: 1 mg

Component	Storage	Amount
A: ReadiUse™ Preactivated PE-iFluor™ 660 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

R-Phycoerythrin (PE) is isolated from red algae. Its primary absorption peak is at 565 nm with secondary peaks at 496 and 545 nm. Our RPE-iFluor™ 660 Tandem is an excellent color for multicolor analysis of multiple targets with flow cytometry. It has a unique emission located between the emission Cy5 and that of Cy5.5, providing a new color for spectral flow cytometry. ReadiUse™ Preactivated PE-iFluor™ 660 Tandem is an activated PE protein, and can be easily conjugated to antibodies with much higher conjugation yield than the conventional PE.

AT A GLANCE

Important PE-iFluor™ 660 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-iFluor™ 660 Tandem (provided) to give the desired PE-iFluor™ 660 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-iFluor™ 660 Tandem

1. Reconstitute pre-activated PE-iFluor™ 660 Tandem in 100 µL ddH₂O to 10 mg/mL.

Note Reconstituted pre-activated PE-iFluor™ 660 Tandem is not stable and can not be stored for more than one month.
2. Add pre-activated PE-iFluor™ 660 Tandem directly to MTA-activated target Ab solution at the ratio of 300 µg PE-iFluor™ 660 Tandem/100 µg MTA-activated Ab.
3. Rotate the mixture for 1 - 2 hours at room temperature.

4. The Ab/PE--iFluor™ 660 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-iFluor™ 660 Tandem can be stored at 4 °C for two months.

5. Optional: Ab/PE-iFluor™ 660 Tandem can be further purified through size exclusion chromatography to get better performance.

EXAMPLE DATA ANALYSIS AND FIGURES

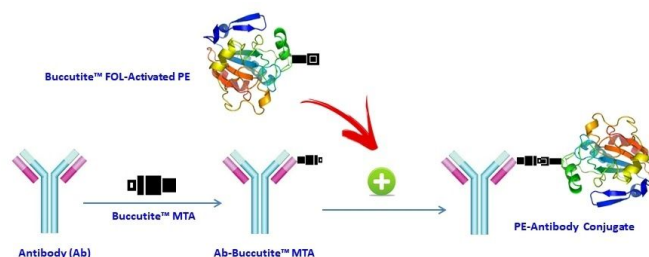


Figure 1. Our preactivated PE-iFluor™ 660 Tandem was premodified with our Buccutite™ FOL (provided). Your antibody (or other proteins) is modified with our Buccutite™ MTA (provided as free sample) to give MTA-modified protein (such as antibody). The MTA-modified protein readily reacts with FOL-modified PE-iFluor™ 660 Tandem (provided) to give the desired PE-iFluor™ 660 Tandem-antibody conjugate in much higher yield than the SMCC chemistry. In addition our preactivated PE-iFluor™ 660 Tandem reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

DISCLAIMER

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