

ReadiUse™ Preactivated PerCP-Cy5.5 Tandem

 Catalog number: 2595
 Unit size: 1 mg

Component	Storage	Amount
A: ReadiUse™ Preactivated PerCP-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

PerCP (Peridinin-chlorophyll-protein complex) is isolated from Dinophyceae sp. It has an extremely high extinction coefficient, a high quantum efficiency and a large Stokes shift. It is well excited with the Argon laser at 488 nm with its maximum emission peak at 677 nm. PerCP protein is commonly used for fluorescent immunolabeling, particularly in applications involving fluorescent-activated cell sorting (FACS). Its tandem conjugates (such as PerCP-Cy5.5) can be excited with a standard 488 nm laser and emits in the far red at a longer wavelength for multicolor flow cytometric analysis of cells. These multiple emission wavelengths make PerCP- Cyanine conjugates potentially useful fluorochromes for multicolor analysis with FITC, PE and other fluorochromes. PerCP tandem structure may make it more photostable than PerCP alone, which generally photobleaches rapidly with more powerful water-cooled gas lasers. AAT Bioquest offers this preactivated PerCP-Cy5.5 Tandem to facilitate the PerCP-Cy5.5 tandem conjugations to antibodies and other proteins such as streptavidin and other secondary reagents. Our preactivated PerCP-Cy5.5 tandem is ready to conjugate, giving much higher yield than the conventionally tedious SMCC-based conjugation chemistry. In addition, our preactivated PerCP-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 PerCP-Cy5.5 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 PerCP Cy5.5 (provided) to give the desired PerCP-Cy5.5-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 PerCP-Cy5.5

1. Reconstitute pre-activated PerCP-Cy5.5 in 100 µL ddH₂O to 10 mg/mL.

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

2. Add pre-activated PerCP-Cy5.5 directly to MTA-activated target Ab solution at the ratio of 75 µg PerCP-Cy5.5/100 µg MTA-activated Ab.
3. Rotate the mixture for 1 - 2 hours at room temperature.
4. The Ab/PerCP-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.

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

5. Optional: Ab/PerCP-Cy5.5 can be further purified through size exclusion chromatography to get better performance.

EXAMPLE DATA ANALYSIS AND FIGURES

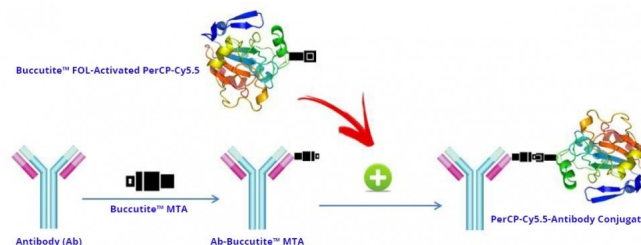


Figure 1. Conjugation scheme for ReadiUse™ Preactivated PerCP-Cy5.5 Tandem. The activated PerCP-Cy5.5 tandem is premodified with our Buccutite™ FOL and can be readily used for conjugation. To conjugate your desired antibody or protein, first modify it with our Buccutite™ MTA (provided) and then mix both components. The Buccutite™ MTA-modified protein will readily react with the Buccutite™ FOL-activated PerCP-Cy5.5 tandem to give the desired PerCP-Cy5.5-antibody conjugate in much higher yield than SMCC chemistry. In addition our preactivated PerCP-Cy5.5 tandem reacts with MTA-modified biopolymers at a much lower concentration than SMCC chemistry.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.