

ReadiUse™ Preactivated APC-iFluor® 750 Tandem

Catalog number: 2571
Unit size: 1 mg

Component	Storage	Amount (Cat No. 2571)
Component A: ReadiUse™ Preactivated APC-iFluor® 750 Tandem	Minimize light exposure, Refrigerated (2-8 °C)	1 vial (1 mg)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µg)

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. Our APC-iFluor® 750 Tandem is an excellent replacement for APC-Cy7® and APC-Alexa Fluor® 750 Tandem due to their similar spectral properties. Our APC-iFluor® 750 Tandem is more photostable than APC-Cy7® Tandem. ReadiUse™ Preactivated APC-iFluor® 750 Tandem is an activated APC protein, and can be easily conjugated to antibodies with much higher conjugation yield than the conventional APC.

SAMPLE EXPERIMENTAL PROTOCOL

Preparation of pre-activated antibody with Buccutite™ MTA

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

Note: Please store unused Buccutite™ MTA at -20 °C and could be used up to two freeze and thaw cycles.

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-iFluor® 750 Tandem

1. Reconstitute pre-activated APC-iFluor® 750 Tandem in 100 µL ddH₂O to 10 mg/mL.

Note: Reconstituted pre-activated APC-iFluor® 750 Tandem could be stored at 4 °C for one month, kept from light.

2. Add APC-iFluor® 750 Tandem directly to MTA-activated target Ab solution at the ratio of 130 µg APC-iFluor® 750 Tandem/100 µg MTA-activated Ab.
3. Rotate the mixture for 60 minutes at room temperature.
4. The Ab/APC-iFluor® 750 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. The Ab/APC-iFluor® 750 Tandem conjugates solution could be stored at 4 °C for up to two months, and kept from light.

5. **Optional:** Ab/APC-iFluor® 750 Tandem conjugates could be further purified through size exclusion chromatography to get best

performance.

EXAMPLE DATA ANALYSIS AND FIGURES

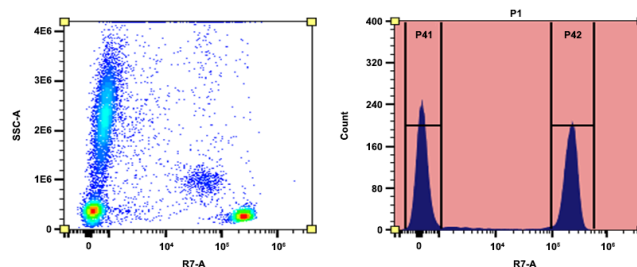


Figure 1. Flow cytometry analysis of whole blood cells stained with APC/iFluor® 750 anti-human CD4 antibody (Clone: SK3). The fluorescence signal was monitored using an Aurora spectral flow cytometer in the APC/iFluor® 750 specific R7-A channel.

DISCLAIMER

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