ReadiUse™ Preactivated APC-iFluor® 705 Tandem

Catalog number: 2573
Unit size: 1 mg

Component	Storage	Amount (Cat No. 2573)
Component A: ReadiUse™ Preactivated APC-iFluor® 705 Tandem	Refrigerated (2-8 °C), Minimize light exposure	1 vial (1 mg)
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µg)

OVERVIEW

ReadiUse™ Preactivated APC-iFluor® 705 Tandem is a preactivated allophycocyanin tandem reagent developed to facilitate conjugation of APC-iFluor® 705 to antibodies and other proteins. APC is a fluorescent phycobiliprotein widely used in flow cytometry because of its strong fluorescence and compatibility with red laser excitation. In this tandem format, APC serves as the donor fluorophore, while iFluor® 705 functions as the far-red acceptor dye for multicolor flow cytometry detection. The preactivated APC-iFluor® 705 tandem is designed for use with Buccutite™ MTA-modified antibodies or proteins. In this workflow, the target protein is first modified with Buccutite™ MTA, and the resulting MTA-modified protein reacts with the FOL-modified preactivated APC tandem to generate the APC-iFluor® 705 protein conjugate. This approach simplifies APC tandem conjugation compared with conventional SMCC-based workflows that require thiol generation by antibody reduction. ReadiUse™ Preactivated APC-iFluor® 705 Tandem is suitable for preparing APC-iFluor® 705-labeled antibodies, streptavidin conjugates, secondary reagents, and other protein probes for flow cytometry applications.

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® 705 Tandem

1. Reconstitute pre-activated APC-iFluor® 705 tandem in 100 µL ddH_2O to 10 mg/mL.

   Note: Reconstituted pre-activated APC-iFluor® 705 tandem could be stored at 4 °C for one month, kept from light.

2. Add APC-iFluor® 705 Tandem directly to MTA-activated target Ab solution at the ratio of 130 µg APC-iFluor® 705 Tandem/100 µg MTA-activated Ab.
3. Rotate the mixture for 60 minutes at room temperature.
4. The Ab/APC-iFluor® 705 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® 705 tandem conjugates solution could be stored at 4 °C for up to two months, and kept from light.

5. **Optional:** Ab/APC-iFluor® 705 Tandem conjugates could be further purified through size exclusion chromatography to get best performance.

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

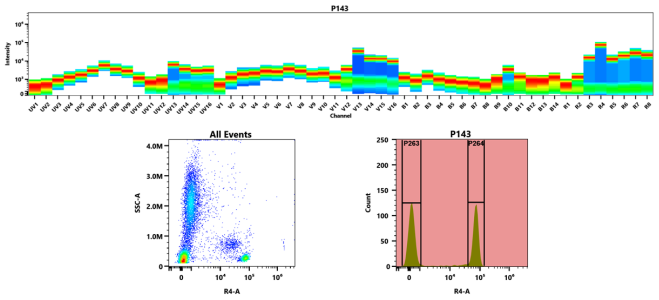


Figure 1. (Top) Spectral emission profiles generated using four spatially offset lasers (355 nm, 405 nm, 488 nm, and 640 nm). Each laser produced a distinct emission pattern, and their combination yielded the composite spectral signature of APC-iFluor® 705. (Bottom) Flow cytometry analysis of whole blood stained with anti-human CD4 antibody (clone SK3; Cat. #10042000) conjugated to APC-iFluor® 705 using Pre-Activated APC-iFluor® 705 Tandem (Cat. #2573) at 0.5 µg/test. The fluorescence signal was monitored using a Cytek Aurora spectral flow cytometer in the R4-A channel.

DISCLAIMER

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