ReadiUse™ Preactivated APC-iFluor™ 700 Tandem

Catalog number: 2570
Unit size: 1 mg

Component	Storage	Amount
A: ReadiUse™ Preactivated APC-iFluor™ 700 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

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. It is a protein which can be easily linked to antibodies and other proteins by conventional protein cross-linking techniques without altering its spectral characteristics. Our APC-iFluor™ 700 Tandem is an excellent replacement for APC-Alexa Fluor® 700 Tandem since they have almost identical spectra. On some antibodies, our APC-iFluor™ 700 Tandem is much brighter than APC-Alexa Fluor® 700 Tandem with a higher stain index. ReadiUse™ Preactivated APC-iFluor™ 700 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™ 700 Tandem

1. Reconstitute pre-activated APC-iFluor™ 700 Tandem in 100 µL ddH_2O to 10 mg/mL.

   Note Reconstituted pre-activated APC-iFluor™ 700 Tandem could be stored at 4 °C for one month, kept from light.

2. Add APC-iFluor™ 700 Tandem directly to MTA-activated target Ab solution at the ratio of 130 µg APC-iFluor™ 700 Tandem/100 µg MTA-activated Ab.

3. Rotate the mixture for 60 minutes at room temperature.

4. The Ab/APC-iFluor™ 700 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™ 700 Tandem conjugates solution could be stored at 4 °C for up to two months, and kept from light.

5. (Optional) Ab/APC-iFluor™ 700 Tandem conjugates could be further purified through size exclusion chromatography to get best performance.

EXAMPLE DATA ANALYSIS AND FIGURES

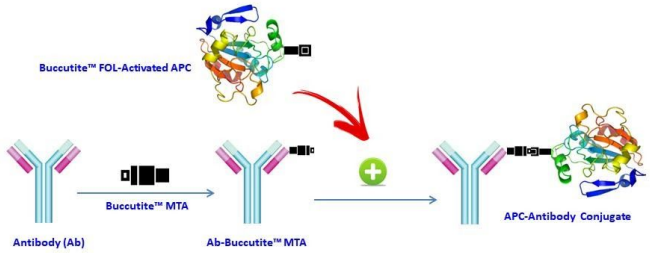

Figure 1. Our preactivated APC-iFluor™ 700 Tandem was premodified with our Buccutite™ FOL (provided). Your antibody (or other proteins) is modified with our Buccutite™ MTA (provided as free sample) to give MTA-modified protein (such as antibody). The MTA-modified protein readily reacts with FOL-modified APC-iFluor™ 700 Tandem (provided) to give the desired APC-iFluor™ 700 Tandem-antibody conjugate in much higher yield than the SMCC chemistry. In addition our preactivated APC-iFluor™ 700 Tandem reacts with MTA-modified biopolymers at much lower concentrations than the SMCC chemistry.

DISCLAIMER

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