

ab312830 – APC-iFluor® 700 Conjugation Kit

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab312830>

Storage and Stability

Upon receipt, store the kit at 4 °C. When stored properly, the kit should be stable for six months. Alternatively, MTA can be stored at -20 °C. Do not freeze FOL-Activated APC-iFluor™ 700 or Reaction Buffer. Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling goat anti-mouse IgG antibody.

Materials Supplied

Item	2 x 25 µg kit	100 µg kit	Storage Condition
FOL-Activated APC-iFluor™ 700	2 vials	2 vials	4°C
MTA	1 vial	2 vials	4°C
Reaction Buffer	1 vial	1 vial	4°C

Reagent Preparation

Antibody working solution:

For labeling 100 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 5 µL (5% of the total reaction volume) of Reaction Buffer with 100 µL of the target antibody solution.

For labeling 25 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 1.25 µL (5% of the total reaction volume) of Reaction Buffer with 25 µL of the target antibody solution.

Note: If you have a different concentration, adjust the antibody volume accordingly to make ~100 µg/ ~25 µg antibody available for your labeling reaction.

Note: The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4; If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use a 10KD Spin Filter to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

Note: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. For BSA removal, we recommend BSA Removal Kit (ab173231). For purification and concentration we recommend Antibody Concentration And Clean-Up Kit (ab102778).

Note: The antibody – MTA reaction efficiency is significantly reduced if the antibody concentration is less than 1 mg/mL. For optimal labeling efficiency the final antibody concentration range of 1-10 mg/mL is recommended.

Assay Procedure

Run Antibody- MTA reaction ~100 µg.

1. Add the antibody working solution directly into the vial of MTA and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Keep the antibody- MTA reaction mixture at room temperature for 30 - 60 minutes.

Note: The antibody- MTA reaction mixture can be rotated or shaken for longer time if desired.

Make antibody-APC-iFluor™ 700 conjugation ~100 µg.

1. Make FOL-Activated APC-iFluor™ 700 solution by adding 50 µL ddH₂O into the vial of FOL-Activated APC-iFluor™ 700, mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix whole vial of FOL-Activated APC-iFluor™ 700 solution into the antibody- MTA solution, mix well and rotating the mixture for 1 hour at room temperature.
3. The antibody-APC-iFluor™ 700 conjugate is now ready to use.

Note: For immediate use, the antibody-APC-iFluor™ 700 conjugate need be diluted with the buffer of your choice.

MTA Working Solution preparation for ~25 µg.

Add 10 µL DMSO (not provided) into the MTA vial.

FOL-Activated APC-iFluor™ 700 Working Solution preparation ~25 µg.

Add 50 µL ddH₂O into the vial of FOL-Activated APC-iFluor™ 700.

Run Antibody- MTA reaction ~25 µg.

1. Add 2.5 µL MTA working solution into antibody working solution and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Keep the antibody- MTA reaction mixture at room temperature for 30 - 60 minutes.

Note: The antibody- MTA reaction mixture can be rotated or shaken for longer time if desired.

Make antibody-APC-iFluor™ 700 conjugation ~25 µg.

1. Add 50 µL of FOL-Activated APC-iFluor™ 700 working solution to the Antibody-MTA solution, mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Incubate for 1 to 2 hours.
3. The antibody-APC-iFluor™ 700 conjugate is now ready to use.

Note: For immediate use, the antibody-APC-iFluor™ 700 conjugate need be diluted with the buffer of your choice.

Storage of Antibody-APC-iFluor™ 700 Conjugate

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). The Antibody-APC-iFluor™ 700 conjugate solution could be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer storage, the antibody-APC-iFluor™ 700 conjugates could be lyophilized and stored at ≤ -20°C.