# Buccutite™ Rapid APC Antibody Labeling Kit

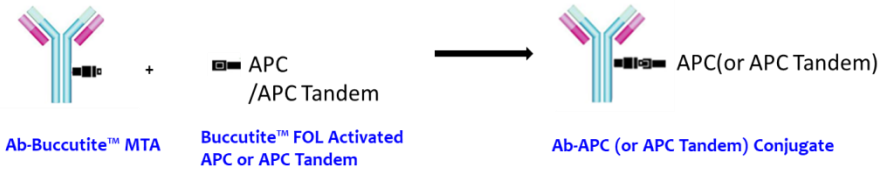
**Microscale Optimized for Labeling 25 ug Antibody Per Reaction**

| *Ordering Information* | *Storage Conditions* |
|---|---|
| Cat#: 1313, 1347, 1350, 1351 (2 conjugations) | Refrigerated |

## Introduction

Protein-protein conjugations are commonly performed with a bifunctional linker (such as the commonly used SMCC), having different reactivity on each end for linking two different proteins. One end of the crosslinker reacts (via NHS ester) with amines (-$NH_2$) found in the amino acid lysine and N-terminus, and the other end reacts (via maleimide) with the thiol groups (-SH) found in the amino acid cysteine. However, SMCC-modified protein is extremely unstable and often self-reactive since proteins often contain both amine and thiol groups that cause significant amount of homo-crosslinking. In addition it is quite difficult and tedious to quantify the number of maleimide groups on a protein.

ReadiLink™ APC Antibody Conjugation Kit is designed for preparing APC or APC tandem conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The APC or APC tandem provided in our kit has been pre-activated with our proprietary linker Buccutite™ FOL, and can be directly used for conjugation. The Buccutite™ FOL -activated APC or APC tandem readily reacts with Buccutite™ MTA-modified molecules under extremely mild neutral conditions without any catalyst required. Compared to commonly used SMCC and other similar technologies, our Buccutite™ bioconjugation system is much more robust and easier to use. It enables faster and quantitative conjugation of biomolecules with higher efficiencies and yields.




## Kit Components

| Components | Amount | Storage |
|---|---|---|
| Component A: Buccutite™ FOL-Activated APC or APC tandem | 2 Vials (lyophilized ) | 4 °C |
| Component B: Buccutite™ MTA | 1 Vial (lyophilized) | 4 °C |
| Component C: Reaction Buffer | 1 Vial (20 µL) | 4 °C (Do not freeze) |

## Standard Operating Protocol (Labeling 25 µg Antibody)

Upon receipt, store the kit at 4 °C. When stored properly, the kit should be stable for six months. Alternatively Components B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated APC (Component A), Reaction Buffer (Component C) and Spin Column (Component D). 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.

### Brief Summary

**Add 1.25 µL reaction buffer (Component C) into antibody (25 µL) → Add 2.5 µL reconstituted Buccutite™ MTA vial (Component B) → Incubate at room temperature → Mix with 50 µL Buccutite™ FOL-Activated APC or APC Tandem (Component A) → incubate at room temperature for 60 min**

**1. Prepare 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 (Component C) with 25 µL of the target antibody solution.

***Note 1****: If you have a different antibody concentration, adjust the antibody volume accordingly to make ~25 µg antibody available for your labeling reaction.*

***Note 2****: 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 Amicon Ultra-0.5, Ultracel-10*

*Membrane, 10 kDa* (*cat# UFC501008 from Millipore*) *to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.*
***Note 3****: Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.*
***Note 4****: The Antibody –Buccutite™ MTA reaction efficiency is significantly reduced if the antibody concentration is lessthan 1 mg/mL. For optimal labeling efficiency the final antibody concentration range of 1-10 mg/mL is recommended.*

**2. Run Antibody-Buccutite™ MTA reaction:**

2.1 Add 10 µL DMSO (not provided in the kit) into the vial of Buccutite ™ MTA (Component B).

2.2 Add 2.5 µL reconstituted Buccutite ™ MTA (Component B), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
*Note: Store reconstituted Buccutite ™ MTA at -20°C, and it should be stable for two weeks with months within one freeze and thaw cycle.*

2.3 Keep the Antibody- Buccutite ™ MTA reaction mixture at room temperature for 30 - 60 minutes.
***Note:*** *The Antibody-Buccutite™ MTA reaction mixture can be rotated or shaken for longer time if desired.*

**3. Make Ab-APC or APC Tandem conjugation:**

3.1 Add 50 µL of $ddH_2O$ into the vial of Buccutite™ FOL-Activated RPE (Component A), gently pipet up and down to mix well.

3.2 Mix whole vial of reconstituted Buccutite™ FOL-Activated APC or APC Tandem (Component A) with the Ab-Buccutite™ MTA solution (from Step 2.3), and rotate the mixture for 1 hour at room temperature.

3.3 The Ab-APC or APC Tandem conjugate is now ready to use.
***Note 1:*** *For immediate use, the Ab-APC or APC Tandem conjugate need be diluted with the buffer of your choice.*
***Note 2:*** *The concentration of the conjugate is about 0.3 mg Ab/mL if start with 25uL 1mg/ml antibody solution.*

## Storage of Ab-APC or APC Tandem Conjugate

The antibody conjugate should be stored in the presence of a carrier protein (e.g., 0.1% bovine serum albumin) and 0.02-0.05% sodium azide. The Ab-APC or APC Tandem conjugate solution could be stored at 4 °C for two months without significant change and kept from light.

## Table 1. Buccutite™ Rapid APC Antibody Labeling Kit (2 Conjugations/Kit, Each Labeling is for 25 µg Antibody)

| Cat. # | Product Name | APC Tandems |
|---|---|---|
| 1313 | Buccutite™ Rapid APC Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody Per Reaction | APC |
| 1347 | Buccutite™ Rapid APC-iFluor™ 700 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug | APC-iFluor™ 700 |
| 1350 | Buccutite™ Rapid APC-Cy5.5 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibody | APC-Cy5.5 |
| 1351 | Buccutite™ Rapid APC-Cy7 Tandem Antibody Labeling Kit *Microscale Optimized for Labeling 25 ug Antibod | APC-Cy7 |

## References

1. Duncan, R.J.S., *et al.* (1983). A new reagent which may be used to introduce sulfhydryl groups into proteins, and its use in the preparation of conjugates for immunoassay. *Anal Biochem* **132:**68-73.
2. Yoshitake, S., *et al.* (1979). Conjugation of glucose oxidase from *Aspergillus niger* and rabbit antibodies using *N*-hydroxysuccinimide ester of *N*-(4-carboxycyclohexyl-methyl)maleimide. *Eur J Biochem* **101:**395-9.
3. Hashida, S., *et al.* (1984). More useful maleimide compounds for the conjugation of Fab' to horseradish peroxidase through thiol groups in the hinge. *J Appl Biochem* **6:**56-63.
4. Imagawa, M., *et al.* (1982). Characteristics and evaluation of antibody- horseradish peroxidase conjugates prepared by using a maleimide compound, glutaraldehyde, and periodate. *J Appl Biochem* **4:**41-57.