Buccutite™ Poly-HRP Antibody Conjugation Kit

Catalog number: 5518, 5519
Unit size: 1x50 ug Labeling, 2x50 ug Labelings

Component	Storage	Amount (Cat No. 5518)	Amount (Cat No. 5519)
Component A: Buccutite™ FOL-Activated Poly-HRP	Refrigerated (2-8 °C), Minimize light exposure	1 vial (200 µL/vial)	2 vials (200 µL/vial)
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	1 vial (Lyophilized)	2 vials (Lyophilized)
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 Vial (20 µL)	1 vial (40 µL)

OVERVIEW

Poly-HRP-antibody conjugates is considered to have the highest sensitivity in immunoassays where the sample is limited, or a target molecule is present at extremely low level. Buccutite™ Poly-HRP Antibody Conjugation Kit is designed for rapid preparation of poly-HRP conjugated antibodies. The kit provides the poly-HRP that is pre-activated with our proprietary linker Buccutite™ FOL. A targeted antibody is activated with Buccutite™ MTA (provided in the kit) to give MTA-activated antibody. The simple mix of MTA-activated antibody with FOL-activated poly-HRP to give the desired poly-HRP-labeled antibody conjugate. The reaction occurs under extremely mild conditions without a catalyst required. The poly-HRP antibody conjugate prepared with Buccutite™ Poly-HRP Antibody Conjugation Kit is compatible with chromogenic, fluorogenic and chemiluminescent HRP substrates used in ELISA, western blotting, immunohistochemistry (IHC) assays. It can also be used with TSA or our Styramide™ fluorescent HRP substrates for ultrasensitive detection of low abundant biological targets.

AT A GLANCE

Protocol summary

1. Add 5 µL of Reaction Buffer (Component C) into antibody (100 µL)
2. Add the antibody solution into the Buccutite™ MTA vial (Component B)
3. Incubate at room temperature for 30 minutes
4. Mix with 200 µL of Buccutite™ FOL-Activated Poly-HRP (Component A)
5. Incubate at room temperature for 60 minutes

Important
Upon receipt, store the kit at 4 °C. When stored properly, the kit should be stable for six months. Alternatively, Component B can be stored at -20 °C. Warm up all the Components to room temperature and centrifuge the vials briefly before opening. Immediately prepare the required solutions before starting your conjugation. The following SOP is an example for labeling 50 µg goat anti-mouse IgG antibody.

PREPARATION OF WORKING SOLUTION

Antibody working solution
For labeling 50 µg antibody (assuming the target antibody concentration is 1 mg/mL), mix 5 µL (5% of the total reaction volume) of Reaction Buffer (Component C) with 50 µL of the target antibody solution.

Note If you have a different concentration, adjust the antibody volume accordingly to make ~50 µg antibody/50 µL 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 10KD Filter (Cat. # 60502 from AAT Bioquest) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate).

Note Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.

Note The antibody –Buccutite™ 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.

SAMPLE EXPERIMENTAL PROTOCOL

Run Antibody-Buccutite™ MTA reaction

1. Add the antibody working solution directly into the vial of Buccutite ™ MTA (Component B), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds.

2. Incubate the antibody-Buccutite ™ MTA reaction mixture at room temperature for 30 minutes.

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

Make HRP-antibody conjugation

1. Add antibody-Buccutite™ MTA reaction mixture to the vial of Buccutite™ FOL-Activated Poly-HRP (Component A), mix well and incubate the mixture at room temperature for 60 minutes.

   Note The antibody poly-HRP reaction mixture can be incubated for longer time if desired.

2. The antibody-poly-HRP conjugate is now ready to use.

   Note For immediate use, the antibody-poly-HRP conjugate needs to be diluted with the buffer of your choice.

3. The concentration of the conjugate can be calculated as follows: **Antibody Concentration (µg/µL) = 50 µg (total amount of antibody)/(50 µL + 5 µL+ 200 µL) = 0.196 µg/µL**

Storage of Antibody-Poly-HRP Conjugate
The antibody conjugate-polyHRP conjugate should be stored in the presence of a carrier antibody (e.g., 0.1% bovine serum albumin) at 4 °C and kept from light for two months.

EXAMPLE DATA ANALYSIS AND FIGURES

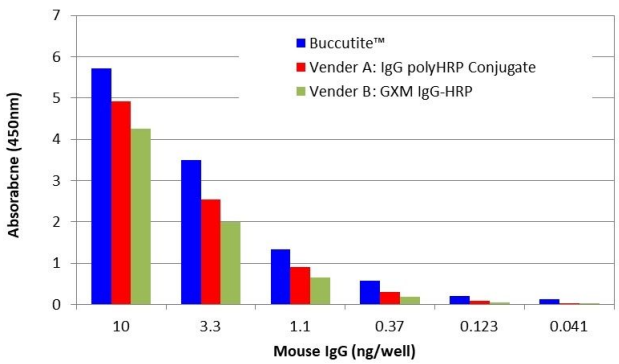

Figure 1. Direct ELISA curves were generated using the polyHRP-goat-anti-mouse IgG conjugate prepared with Buccutite™ Poly-HRP Antibody Conjugation Kit (Cat#5518 or Cat#5519). 3-fold serial diluted mouse IgG was coated on a 96-well plate, and 100uL GAM IgG-polyHRP conjugate (100ng/ml) was tested using the standard ELISA method. TMB substrate solution (Cat#11003) was used to detect the immobilized mouse IgG with 5 min incubation and read at 450 nm.
Blue: 5518 or 5519 Kit
Red: GXM IgG PolyHRP (Vendor A)
Green: GXM IgG-HRP (Vendor B)

DISCLAIMER

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