Buccutite™ Rapid trFluor™ D2 Acceptor Antibody Labeling Kit *Microscale Optimized for Labeling 100 ug Antibody Per Reaction*

Catalog number: 1302
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 1302)
Component A: Buccutite™ FOL-Activated trFluor™ D2	Refrigerated (2-8 °C), Minimize light exposure	2 Vials (Lyophilized)
Component B: Buccutite™ MTA	Refrigerated (2-8 °C), Minimize light exposure	2 Vials (Lyophilized)
Component C: Reaction Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 Vial (20 µL)

OVERVIEW

Many biological compounds present in cells, serum, or other biological fluids are naturally fluorescent, and thus the use of conventional, prompt fluorophores leads to serious limitations in assay sensitivity due to the high background caused by the autofluorescence of the biological molecules to be assayed. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences. Our trFluor™ Eu probes enable time-resolved fluorometry (TRF) for the assays that require high sensitivity. These trFluor™ Eu probes have large Stokes shifts and extremely long emission half-lives when compared to traditional fluorophores such as Alexa Fluor or cyanine dyes. Compared to the other TRF compounds, our trFluor™ Eu probes have relatively high stability, high emission yield, and the ability to be linked to biomolecules. Buccutite™ Rapid trFluor™ D2 Acceptor Antibody Labeling Kit provides a fast way to prepare the D2 acceptor-labeled bioconjugates that are used to pair to the trFluor™ Eu-labeled probes to develop TR-FRET assays.

AT A GLANCE

Protocol Summary

1. Add 5 µL Reaction Buffer (Component C) into the antibody solution (100 µL).
2. Add the antibody solution into Buccutite™ MTA vial (Component B).
3. Incubate at room temperature for 30 minutes.
4. Mix with 50 µL Buccutite™ FOL-Activated trFluor™ D2 working solution.
5. Incubate at room temperature for 60 minutes.

Important Note

Please store the kit at 4°C upon receiving it. Ensure it is stored properly to maintain stability for six months. Alternatively, Component B can be stored at -20°C. Do not freeze Buccutite™ FOL-Activated trFluor™ D2 (Component A) or Reaction Buffer (Component C). Before opening the vials, warm all components and briefly centrifuge them. Prepare the required solutions immediately after opening the vials to begin your conjugation. For reference, an example SOP for labeling goat anti-mouse IgG antibodies is provided.

PREPARATION OF WORKING SOLUTION

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 (Component C) with 100 µL of the target antibody solution.

Note: If you have a different concentration, adjust the antibody volume accordingly to make ~100 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 ReadiUse™ 10KD Spin Filter (Cat. 60502 from AAT Bioquest) 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.

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. 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 a longer time if desired.

Make antibody-trFluor™ D2 conjugation

1. Make Buccutite™ FOL-Activated trFluor™ D2 solution by adding 50 µL ddH2O into the vial of Buccutite™ FOL-Activated trFluor™ D2 (Component A), mix well by repeatedly pipetting for a few times or vortex the vial for a few seconds.
2. Mix the whole vial of Buccutite™ FOL-Activated trFluor™ D2 solution into the antibody-Buccutite™ MTA solution, mix well, and rotate the mixture for 1 hour at room temperature.
3. The antibody-trFluor™ D2 conjugate is now ready to use.

   Note: For immediate use, the antibody-trFluor™ D2 conjugate needs to be diluted with the buffer of your choice.

Storage of Antibody-trFluor™ D2 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-trFluor™ D2 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-trFluor™ D2 conjugates could be lyophilized and stored at ≤ –20 °C.

EXAMPLE DATA ANALYSIS AND FIGURES

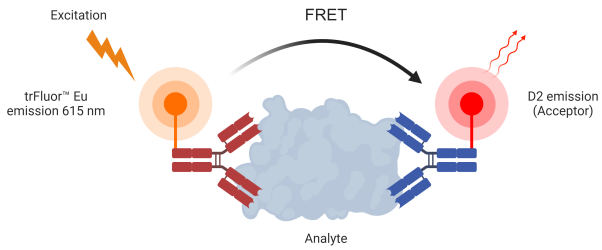

Figure 1. D2 acceptor is used to pair with Eu-labeled probes (such as TR Fluor™ Eu) for developing TR-FRET assays. TR-FRET assays are much more sensitive than the regular FRET assays that suffer serve interference caused by the naturally fluorescent compounds present in cells, serum or other biological fluids. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes prompt fluorescence interferences.

DISCLAIMER

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