Buccutite™ FOL-Dye 650

Catalog number: 5372
Unit size: 2 umoles

Component	Storage	Amount
Buccutite™ FOL-Dye 650	Freeze (<-15 °C), Minimize light exposure	2 umoles

OVERVIEW

Our Buccutite™crosslinking technology provides the most convenient and effective crosslinking method to link two biomolecules with a high conjugation yield. Our method uses one pair of crosslinkers: Buccutite™ MTA and Buccutite™ FOL. MTA is added to one molecule, while FOL is added to another molecule. The cross-linking reaction is initiated by mixing Molecule-1-Buccutite ™ MTA and Molecule-2-Buccutite ™ FOL. This crosslinking reaction occurs under extremely mild and neutral conditions without any catalyst required. It is robust and efficient. Many of our customer have requested us to offer the stand-alone Buccutite™ MTA and Buccutite™ FOL reagents to expand the application of Buccutite™crosslinking technology. This Buccutite™ FOL reagent is used to determine the number of FOL groups of the Molecule-2-Buccutite ™ FOL. The number of FOL linkers provides an important parameter to to optimize crosslinking process.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Buccutite™ FOL-Dye 650 stock solution (10 mM):
Add 200 uL DMSO to Buccutite™ FOL-Dye 650 vial to prepare 10 mM stock solution.

Note The Buccutite™ FOL-Dye 650 stock solution should be stored at -20 °C after preparation and stable for 2 months if avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

FOL Sample Preparation

1. Use 100 ug FOL-modified sample (for example: antibody or other protein modified with MTA group, the MW should be above 15,000).
2. Adjust the volume to 100 uL with PBS.

Run FOL Assay

1. Add 10 uL 10 mM Buccutite™ FOL-Dye 650 stock solution to FOL sample solution.
2. Keep the reaction mixture at room temperature and rotate or shake it for 60 minutes.
3. Prepare spin column (Cat#60500) for sample purification.
4. Load the reaction mixture to a spin column with a clean collecting tube. After all the solution loaded to the column, add 10 uL PBS to the top and centrifuge the column for 5 minutes at 1,000 x g.
5. Collect the solution with a collecting tube.
6. Measure the absorption spectra with 0.5 mL Quartz Cuvette or Nanodrop.

   Note Dilute the elution by 5 - 10 folds with PBS, measure the absorption spectrum from 800 nm to 250 nm, or only read the absorbance number at 280 nm and 654 nm.

7. Calculate FOL # (moles of FOL / mole of molecule) with the following equation.

FOL # = (A654 / 250000) / {(A280 - 0.09 X A654) / EC}

A280: absorbance of the elution at 280 nm
A654: absorbance of the elution at 654 nm
EC: Extinction Coefficient of the sample ($M^{-1}cm^{-1}$)

EXAMPLE DATA ANALYSIS AND FIGURES

FOL Calculations:

Sample: GxM IgG-FOL, 100 ug in 100 uL PBS

Measure absorbance with a Nanodrop spectrophotometer,

A280 nm = 0.766,
A654 nm = 0.852,
CF280 = 0.09,
EC (Buccutite™ FOL-Dye 650) at 650 nm = 250,000 M^{-1} cm^{-1}
EC of IgG at 280 nm = 210,000 M^{-1} cm^{-1}

FOL # (moles of MTA per mole of IgG) = (0.852 / 250000) / {(0.766 - 0.09 X 0.852) / 210000} = 1.0

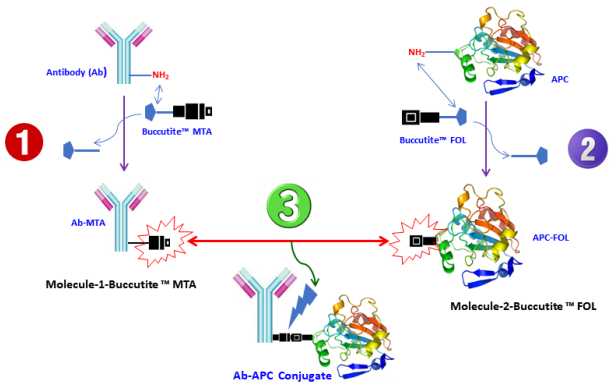

Figure 1. Buccutite™crosslinking technology provides the most convenient and effective crosslinking method to link two biomolecules with a high conjugation yield. Our method uses one pair of crosslinkers: Buccutite™ MTA and Buccutite™ FOL. MTA is added to one molecule, while FOL is added to another molecule. The cross-linking reaction is initiated by mixing Molecule-1-Buccutite ™ MTA and Molecule-2-Buccutite ™ FOL. This crosslinking reaction occurs under extremely mild and neutral conditions without any catalyst required. It is robust and efficient.

DISCLAIMER