Buccutite™ FOL, NHS ester

Catalog number: 5350
Unit size: 2 umoles

Component	Storage	Amount
Buccutite™ FOL, NHS ester	Freeze (< -15 °C), Minimize light exposure	1 vial (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. A number of our customers have requested us to offer the stand-alone Buccutite™ MTA and Buccutite™ FOL reagents to expand the application of Buccutite™crosslinking technology. Protein-protein conjugations are commonly performed with a bifunctional linker such as SMCC. One end of the SMCC reacts (via NHS ester) with amines (-NH2) 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.

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, NHS ester stock solution (20 mM)
Add 100 µL high-quality, anhydrous dimethylsulfoxide (DMSO) or dimethyl-formamide (DMF) to Buccutite™ FOL, NHS ester vial to prepare 20 mM stock solution.

Note Dissolve the dye immediately before starting the reaction.

Note The Buccutite™ FOL, NHS ester stock solution should be stored at -20 °C after preparation. Once reconstituted, the NHS ester reactive dye solution is not very stable, especially if exposed to moisture. It could hydrolyze into the nonreactive free acid in aqueous solutions.

SAMPLE EXPERIMENTAL PROTOCOL

1. Prepare protein working solution:For labeling 1 mg protein (assuming the concentrations are 10 mg/mL), prepare protein solution in pH=8.5 buffer by buffer exchange.

   Note The Buccutite™ FOL modification reaction is highly concentration dependent, the protein concentration should usually be 2-10 mg/mL. Concentrations lower than 2 mg/mL will greatly decrease the efficiency of the reaction.

   Note Protein solutions must be free of any amine-containing substances such as Tris, glycine, ammonium ions, or stabilizing proteins such as bovine serum albumin. You can dialyze protein against 10 mM phosphate-buffered saline (1x PBS), and then add 5% (v/v) of 1 M sodium bicarbonate buffer (pH 8.5–9.0) to antibody solution to adjust pH to ~8.5.

   Note The presence of low concentrations of sodium azide (<3 mM) or thimerosal (<1 mM) will not interfere with the conjugation reaction.

2. Add the amount of Buccutite™ FOL, NHS ester stock solution needed to the protein solution, mix well and incubate the reaction for 1 hour at room temperature with continuous stirring.

   Note Please modify the dye-to-protein labeling ratio used in the reaction to achieve the desired number.

3. Purification the reaction mixture with desalting column. Purify the conjugate by column chromatography with PBS buffer or other buffer of choice.

   Note If the reaction volume is ~100 µL, spin column (Cat# 60500) can be used to purify. If the reaction volume is > 1 mL, we recommend packing a column using Sephadex® G-25 media or Bio-Gel® P-6DG Gel with an appropriate volume to purify the reaction mixture.

4. Collect the purified protein/FOL solution.

5. Determine the protein concentration:FOL linker has very low absorbance at 280 nm, measuring absorbance is an easy way to determine protein concentration if the protein extinction coefficient is known. BCA assay or other protein assay is compatible with FOL modified protein.

Determine the number of FOL groups:
The number of FOL groups can be determined with Buccutite™ FOL-Dye 650 (Cat#5372).
Table 1. Here is a reference for The number of FOL groups after Buccutite™ FOL modification reaction:

Antibody Concentration*	Labeling Ratio**	FOL numbers / IgG molecule
GXM IgG, 2 mg/ml	10:1	2.5~3.0
GXM IgG, 10 mg/ml	3:1	1.0~1.5
GXM IgG, 10 mg/ml	5:1	2.0~2.5
GXM IgG, 10 mg/ml	10:1	3.0~4.0

*: GxM IgG solution at specified concentration, pH~8.5, 100 uL /reaction
**: Molar ratio of Buccutite™ FOL, NHS and antibody during the reaction

Storage of Buccutite™ FOL modified protein:
Buccutite™ FOL modified protein conjugate can be temporarily stored in PBS at 2~8°C and avoid light for ~2 weeks without significant decrease of FOL number. For long term storage, the Buccutite™ FOL modified protein could be lyophilized the same way as the unmodified protein, and stable for a year without significant decrease of FOL reactivity.

EXAMPLE DATA ANALYSIS AND FIGURES

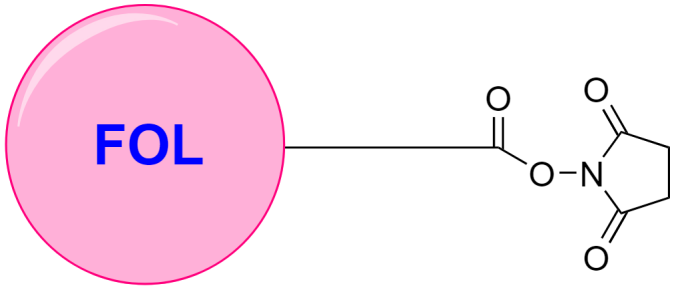

Figure 1. Chemical structure for Buccutite™ FOL, NHS ester.

DISCLAIMER

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