Buccutite™ Rapid Oligo Antibody Conjugation Kit *Optimized for Labeling 100 ug Protein*

Catalog number: 5450
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5450)
Component A: Buccutite™ FOL	Freeze (< -15 °C), Minimize light exposure	2 vials
Component B: Buccutite™ MTA	Freeze (< -15 °C), Minimize light exposure	2 vials
Component C: Reaction Buffer	Refrigerated (2-8 °C)	1 vial (100 µL)
Component E: Spin Column	Refrigerated (2-8 °C)	2 columns

OVERVIEW

Buccutite™ Rapid Oligo Antibody Conjugation Kit is optimized for conjugating 100 ug protein with an amino-modified oligo. The oligonucleotide conjugation kit enables the easy and efficient generation of antibody-oligonucleotide conjugates. The Buccutite crosslinking technique has been proven to be one of the most effective conjugation methods for crosslinking two large molecules. The kit is one of the most effective oligo-antibody conjugation products. It can be used to generate conjugates of different ratios of antibody/oligonucleotide. It can be used to conjugate antibodies to single-stranded or double-stranded oligonucleotides that are modified with an amine group, typically on a terminal. It is quick and easy to use with high levels of antibody and oligo recovery. It is flexible to control the ratio of oligo/antibody at your desired level. The resulting conjugate is highly stable since the oligo and antibody are covalently connected via the highly stable amide bond. It can be used for a wide range of target proteins, including antibody fragments and small proteins. Antibody-oligonucleotide conjugates (AOCs) belong to a class of chimeric molecules combining two important families of biomolecules: antibodies and oligonucleotides. Combination of exceptional targeting capabilities of antibodies with numerous functional modalities of oligonucleotides has been fruitful for a variety of applications with AOC including single cell sequencing, imaging, detection and targeted therapeutics.

AT A GLANCE

Key Parameters to Achieve Best Performance:

1. Protein molecular weight: >25,000 Daltons
2. Protein concentration: >=1mg/mL
3. Protein sample volume: 60~120 µL

PREPARATION OF WORKING SOLUTION

Prepare Protein Solution

1. To label 100 µg protein (assuming that the concentration is 1 mg/mL in PBS), mix 5 µL (5% of the total reaction volume) of Reaction Buffer (Component C) with 100 µL of the protein solution.

   Note: The protein should be dissolved in 1X phosphate-buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, or use ReadiUse™ 10KD Spin Filter (Cat No. 60502) to remove free amines or ammonium salts, such as ammonium sulfate and ammonium acetate, that are widely used for protein precipitation.

   Note: Impure proteins or proteins stabilized with bovine serum albumin (BSA) or gelatin will not label well.

Prepare Oligo Solution

1. To label 200 µg of an amine-labeled oligo (assuming a concentration of 2 mg/mL), mix 5 µL (5% of the total reaction volume) of the Reaction Buffer (Component C) with 100 µL of the oligo solution.

   Note: The oligo should be dissolved in nuclease-free water for better results.

SAMPLE EXPERIMENTAL PROTOCOL

Run Protein-Buccutite™ FOL Reaction

1. Add 105 µL of the protein solution directly to the vial of Buccutite™ FOL (Component A). Mix well by pipetting or vortexing.
2. Keep the protein-Buccutite™ FOL reaction mixture at room temperature for 30 - 60 minutes.

   Note: The protein-Buccutite™ FOL reaction mixture can be rotated or shaken for a longer time if desired.
3. Purify the protein-Buccutite™ FOL reaction mixture using the desalting column. Please refer to the 'Prepare spin column for Antibody purification' section below for detailed instructions.
4. Calculate the concentration of the protein-Buccutite™ FOL mixture with 75% yield after desalting. (For example: if starting with 100 µg protein, after desalting column purification, the recovery protein amount is ~75µg.)

Run Oligo-Buccutite™ MTA Reaction

1. Add 105 µL of the amine-labeled oligo solution directly to the vial of Buccutite™ MTA (Component B). Mix well by pipetting or vortexing.
2. Keep the oligo-Buccutite™ MTA reaction mixture at room temperature for 30 - 60 minutes.

   Note: The oligo-Buccutite™ MTA reaction mixture can be rotated or shaken for a longer time if desired.
3. Purify the oligo-Buccutite™ MTA reaction mixture using alcohol precipitation. Please refer to the 'Purification of Oligo with Alcohol Precipitation' section below for detailed instructions.

Run Protein-Oligo Conjugation Reaction

1. To start the conjugation reaction, mix the protein-Buccutite™ FOL reaction mixture with the oligo-Buccutite™ MTA reaction mixture at the desired molar ratio.

   Note: You can adjust the mixing ratio depending on your downstream applications and the cost of your proteins.
2. Rotate the mixture for 1-2 hours at room temperature or at 4°C

overnight. The reaction mixture is ready for immediate use or can be stored at 4°C.

Note: Desalting is optional.

Optional: Conjugate Analysis and Purification

1. A small amount of the reaction mixture, such as 2-4 µg, can be analysed using a 4-12% Bis-Tris Protein Gel in an SDS running buffer system to verify the conjugation outcome.

2. The conjugation reaction mixture contains both the desired conjugate and a small amount of unconjugated oligo. If required, the reaction mixture can be purified by size exclusion chromatography (SEC), and the desired conjugate fractions can be pooled and combined.

Prepare Spin Column for Protein Purification

1. Invert the provided spin column (Component D) several times to re-suspend the settled gel and remove any bubbles.

2. Snap off the tip and place the column in a washing tube (2 mL, not provided). Remove the cap to allow the excess packing buffer to drain by gravity to the top of the gel bed.

   Note: If the column does not begin to flow, push the cap back into the column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Washing Tube.

3. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer. Refer to the 'Centrifugation Notes' section below for instructions.

4. Apply 1-2 mL 1X PBS (pH 7.2-7.4) to the column. After each application of PBS, let the buffer drain out by gravity, or centrifuge the column for 2 minutes to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.

5. Centrifuge at 1000 x g for 2 minutes in a swinging bucket centrifuge to remove the packing buffer. Then discard the buffer. Refer to the 'Centrifugation Notes' section below for detailed instructions.

6. Place the column into a clean collecting tube (1.5 mL, not provided). Then, take 105 µL of the protein-Buccutite™ FOL reaction mixture from step 2 of the "Run Protein-Buccutite™ FOL Reaction" section and load it carefully and directly into the center of the column.

7. After loading the sample, add 10 µL of 1X PBS (pH 7.2-7.4), centrifuge the column for 5-6 minutes at 1,000 x g, and collect the solution that contains the desired protein-Buccutite™ FOL solution.

Purification of Oligo with Alcohol Precipitation

1. Add 0.1 volume (10 uL) of 5M sodium chloride and 2 - 2.5 volumes of ice-cold 100% ethanol (250 uL) to the oligo-Buccutite™ MTA reaction mixture. Mix well and place at ≤ -20°C for at least 30 minutes.

2. Centrifuge at full speed (>14,000 x g) in a refrigerated micro centrifuge for 15-30 minutes to pellet the labeled nucleic acid. Once pelleted, gently remove the ethanol with a micropipette; do not disturb the pellet.

   Note: Small nucleic acid quantities can be difficult to visualize. Mark and orient the precipitate-containing tubes in the microfuge such that the pellet will form in a predetermined place.

3. Wash the pellet once with 500 µL of room temperature 70% ethanol. Centrifuge at full speed for an additional 15-30 minutes.

4. Remove all traces of ethanol with a micropipette. DO NOT allow the sample to dry beyond 5 minutes as the pellet may become difficult to re-suspend.

5. Re-suspend the labeled oligo with sterile water (we recommended adding 30 uL of water).

6. Store the purified, labeled oligo on ice for immediate use.

Centrifugation Notes

1. The spin column (Component D) can fit into a 2 mL microcentrifuge tube or 12 x 75 mm test tube for sample collection during centrifugation. Use the 2 mL microtube with the columns for the initial column equilibration step.

2. Swinging bucket centrifuges are capable of generating a minimum force of 1,000 x g and are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge rotor. Consult the swinging bucket centrifuge instruction manual for information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.

3. Alternatively, use the following equation to calculate the speed in RPM required to reach the gravitational force of 1,000 x g:

$$\mathbf{RCF\ (x\ g) = (1.12 \times 10^{-5}) \times (RPM) \times 2 \times r}$$

   - **RCF** is the relative centrifugal force.
   - **r** is the radius in centimeters measured from the center of the rotor to the middle of the Bio-Spin column.
   - **RPM** is the speed of the rotor.

EXAMPLE DATA ANALYSIS AND FIGURES

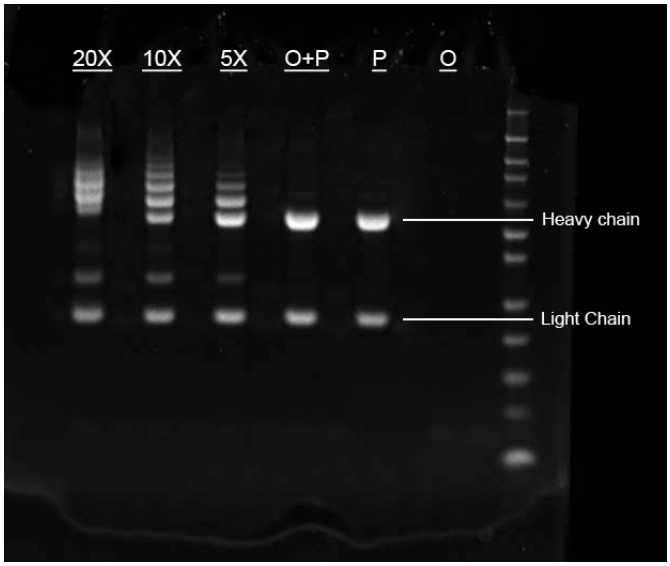

Figure 1. The Buccutite™ Rapid Oligo Antibody Conjugation kit (AAT Cat# 5450) was utilized to conjugate rabbit IgG with a 20-nucleotide-

containing oligo. The conjugation was confirmed using a 4-12% reducing gel SDS-PAGE. The reaction mix ratios of antibody:oligo examined were 5X, 10X, and 20X, as well as the reactions of oligo + protein (O+P), protein alone (P), and oligo alone (O).

DISCLAIMER

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