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Link-A-Light Glucose Oxidase Conjugation Kit (1 labeling reaction)

Catalog No.: AC003-003

Quantity: 1 x 1 mg

Reaction: 1 labeling reaction

Kit Contents:

AC003-003-M1:	Link-A-Light Mix (Glucose Oxidase) (Ready to Use)
AC003-003-R1:	Link-A-Light Modifier reagent (Ready to Use)
AC003-003-Q1:	Link-A-Light Quencher reagent (Ready to Use)

Conjugate: Glucose Oxidase

Background: Link-A-Light is a one step antibody labeling kit, requiring just 30 seconds hands-on time. The antibody is covalently bonded to the label in a directional and controlled process at near-neutral pH.
The Link-A-Light conjugation kit allows Glucose Oxidase (GOx) conjugations to set up in seconds, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing GOx from Aspergillus niger (Figure 1).
By circumventing the desalting or dialysis steps that commonly interrupt traditional protein conjugation procedures, Link-A-Light technology can be used to label small quantities of protein (see Principle).

Label: Glucose Oxidase is an enzyme produced and purified from Aspergillus niger which catalyses the oxidation of glucose with the release of hydrogen peroxide. It's molecular weight is 80kDa, but it exists as a dimer of 160kDa Measurement of glucose by GOx is used in the food industry, in fermentation and, most importantly, as the basis of biosensors for the diagnosis of diabetes.

Applications: Labeling

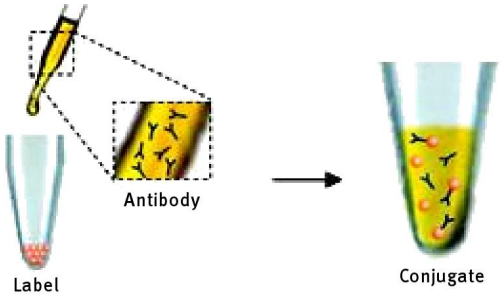

Figure 1

Principle of the Procedure: Upon dissolution of Link-A-Light mixture with a solution of the antibody (or other biomolecule to be labeled) proprietary chemicals in the mixture become activated. This results in the directional, covalent bonding of the antibody to the enzyme label in a gentle

and controlled process at near-neutral pH. The hands-on time for the entire procedure is usually 20-30 seconds. Link-A-Light makes it possible to label antibodies and other biomolecules with GOx with ease, and eliminates the need for secondary detection reagents. Direct labeling of antibodies simplifies and shortens immunoassay procedures and generally improves data quality.

Frequently asked Questions:

Q1. What functional groups do I need on my protein? Link-A-Light requires amine groups on the molecule to be labeled. Most proteins have lysine and/or alpha-amino groups. All antibodies will have multiple amine functions. **Q2.** Do I need to purify the conjugate? No. The chemicals used in Link-A-Light are deactivated by the Quencher, and the by-products are benign and do not need to be removed.

Materials Required but Not Provided:

Antibody to be Labeled: The best ratio for any new antibody reagent must be determined by experimentation but 80-100 µg of IgG antibody for every 100 µg of Link-A-Light GOx usually gives optimal results. The 100 µg quantity of antibody corresponds to an Ab:GOx molar ratio of 1:1 (Mw of GOx is 160kDa). The volume in which the antibody is added ideally should be around 4-10 µl (10 µg pack size), 40-100 µl (100 µg pack size) and 400-1000 µl (1mg pack size). **Sample Buffer:** Ideally, the antibody to be labeled should be in 10-50mM amine-free buffer pH range 6.5 to 8.5. However, many buffers outside these limits of concentration and pH can be accommodated. Modest concentrations of Tris buffer are also tolerated. **Compatibility of Buffers and Buffer Additives:** Amine-free buffers, including MES, MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 6.5-8.5, as the addition of Link-A-Light Modifier provides the conditions necessary for efficient conjugation. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA) and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect. If the amine-free buffer is relatively concentrated and outside the pH range 6.6-8.5 you may need to add more Link-A-Light Modifier for each 10 µl of antibody. Excess Link-A-Light Modifier is provided so that you can check the pH of the buffer after the addition of the Modifier. Ideally the pH should be around 7.3-7.6, though efficient conjugation occurs anywhere between pH 6.8 and 7.8. Avoid buffer components that are nucleophilic, as these may react with Link-A-Light chemicals. Primary amines (e.g. amino acids, ethanolamine) and thiols (e.g. mercaptoethanol, DTT) fall within this class. Note: Unusually for an amine, Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less.

Protocols:

1. Before you add antibody to the Link-A-Light Mix, add 1 µl of Link-A-Light Modifier reagent for each 10 µl of Antibody to be labeled. Mix gently.
2. Remove the screw cap from the vial of Link-A-Light Mix and pipette the antibody sample (with added Link-A-Light Modifier) directly onto the lyophilised material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.
3. Place the cap back on the vial and leave the vial standing for 3 hours at room temperature (20-25°C). Alternatively, and sometimes more conveniently, conjugations can be set up and left overnight, as the longer incubation time has no negative effect on the conjugate.
4. After incubating for 3 hours (or more), add 1 µl of Link-A-Light Quencher FD reagent for every 10 µl of antibody used. The conjugate can be used after 30 minutes.

Storage:

Storage of Conjugates: For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for Long-term storage. Other storage conditions (e.g. Frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

Storage of Kit: The kit is shipped at ambient temperature in a tamper-evident polypropylene container. Store at -20°C upon receipt.

License:

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