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Link-A-Light Biotin Conjugation Kit (Type B) (3 labeling reactions, each 100-200 µg)

Catalog No.: AC044-002

Quantity: 1 kit

Reaction: 3 labeling reactions, each 100-200 µg

Kit Contents:

AC044-002-M1:	Link-A-Light Mix (Ready to Use)
AC044-002-R1:	Link-A-Light Modifier reagent (Ready to Use)
AC044-002-Q1:	Link-A-Light Quencher reagent (Ready to Use)

Conjugate: Biotin

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 Biotinylations to be set up in **seconds**, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing a proprietary activated Biotin Ligand (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 with 100% recovery (see Principle).
Link-A-Light Biotin has been optimised for two separate applications.
Type A is intended for assays in which a Streptavidin-labeled detection reagent will be used, whilst **Type B** is optimised for assays in which the biotinylated protein is captured by Streptavidin immobilized on a surface (ie plates, nitrocellulose, magnetic beads etc).

Label: The attachment of Biotin to biomolecules is an important laboratory technique. Biotin binds to the tetrameric avidin proteins, including streptavidin and neutravidin, with exceptionally high affinity, and this interaction is exploited in various applications such as Western blotting, Immunohisthochemistry and ELISA. The Biotin in the kit has an extended linker to facilitate molecular interactions.

Applications: Labeling

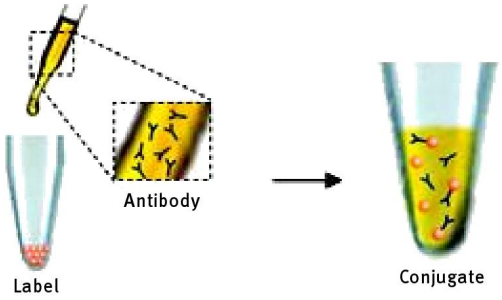

Figure 1

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 coupling the antibody to the Biotin which has an extended spacer, in a gentle and controlled process at near-neutral pH. Link-A-Light makes it possible to biotinylate primary antibodies and other proteins with ease, using a simple, efficient process that has safeguards to prevent over-labeling of the biomolecule and that ensures 100% recovery even at small scale.

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. Moreover, the conjugation efficiency is very high and, unlike other biotin reagents, Link-A-Light Biotin does not hydrolyze in solution or during storage, thus it is not necessary to use a large excess or to purify the final conjugate.

Materials Required but Not Provided:

Antibody to be Labeled: The recommended amount of antibody to be used for labeling is up to 20 µg for **AC044-001**, 200 µg for **AC044-002** and up to 2 mg for **AC044-003**. The volume of the antibody sample, ideally, should be in the range 4-10 µl for **AC044-001**, 40-100 µl for **AC044-002** and 400-1000 µl for **AC044-003**. Antibody concentrations of 1 mg/ml or greater generally gives the best results. The Link-A-Light Biotin kit contains a stable form of Biotin and is much more efficient that standart Biotin kit, thus **no purification** of the conjugate is required (see also **Q2**) **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.

Add. Information: **Note:** All of the components in the kit are compatible with our range of Link-A-Light one-step conjugation kits. This means that you can do direct from purification to labeling.

Storage: **Storage of Conjugates:** For any new conjugate, 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.

General Readings:

1. Morgan R, Boxall A, Bhatt A, Bailey M, Hindley R, Langley S, et al. Engrailed-2 (EN2): a tumor specific urinary biomarker for the early diagnosis of prostate cancer. Clin Cancer Res. 2011 Mar 1;17(5):1090-8. doi: 10.1158/1078-0432.CCR-10-2410. Epub 2011 Mar 1. PubMed PMID: 21364037.
2. Gijsen M, King P, Perera T, Parker PJ, Harris AL, Larijani B, et al. HER2 phosphorylation is maintained by a PKB negative feedback loop in response to anti-HER2 herceptin in breast cancer. PLoS Biol. 2010 Dec 21;8(12):e1000563. doi: 10.1371/journal.pbio.1000563. PubMed PMID: 21203579.
3. Sarugaser R, Hanoun L, Keating A, Stanford WL, Davies JE. Human mesenchymal stem cells self-renew and differentiate according to a deterministic hierarchy. PLoS One. 2009 Aug 4;4(8):e6498. doi: 10.1371/journal.pone.0006498. PubMed PMID: 19652709.

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