AC046-002

**OriGene Technologies Inc.**

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

**OriGene EU**

**Acris Antibodies GmbH**
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

# Link-A-Light Avidin Conjugation Kit (3 labeling reactions)

**Catalog No.:** AC046-002

**Quantity:** 3 x 100 µg

**Reaction:** 3 labeling reactions

**Kit Contents:**

| | |
|---|---|
| AC046-002-M1: | Link-A-Light Mix (Ready to Use) |
| AC046-002-R1: | Link-A-Light Modifier reagent (Ready to Use) |
| AC046-002-Q1: | Link-A-Light Quencher reagent (Ready to Use) |

**Conjugate:** Avidin

**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 Avidin conjugations to be set up in **seconds**, simply by adding a solution of the antibody of the protein to be labeled to a proprietary lyophilised mixture containing a proprietary activated Avidin 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).
**Label:** Avidin is a 66kDa tetrameric glycoprotein composed of four identical subunits, which has widespread applications due to its very high affinity to the vitamin Biotin.

**Applications:** Labeling

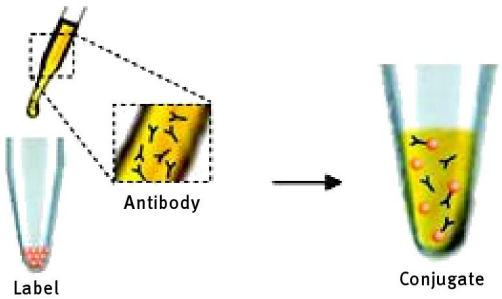


**Figure 1**

**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 Avidin, 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 and do not need to be removed. **Q3.** Can Non antibody molecules be labeled? Yes. While labeling of antibodies is a major application, the only requirement is that the protein to be labeled has Amine functionality.

**Materials Required but Not Provided:**

**Antibody to be Labeled:** The amount of antibody used for labeling should be the same as the pack size of Link-A-Light Avidin. e.g. For 100 µg Link-A-Light Avidin add 100 µg of antibody. The volume in which the antibody is added, ideally, should be in the range 4-10 µl for **AC046-001**, 40-100 µl for **AC046-002** and 400-1000 µl for **AC046-003**. Antibody concentrations in the range 1-4 mg/ml are therefore ideal. However, concentrations and volumes outside these suggested limits have also yielded good conjugates. For any new antibody, optimization of the ratio of antibody to Avidin is often worthwhile. **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, 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. Targeting MHC Class I Monomers to Dendritic Cells Inhibits the Indirect Pathway of Allorecognition and the Production of IgG Alloantibodies Leading to Long-Term Allograft

Survival. Yakup Tanriver, Kulachelvy Ratnasothy, R. Pat Bucy, Giovanna Lombardi and Robert Lechler.
Journal of Immunology, February 15, 2010 vol. 184 no. 4 1757-1764.

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