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 ATTO633 Conjugation Kit (1 labeling reaction, 1-2 mg)

Catalog No.: AC025-003

Quantity: 1 kit

Reaction: 1 labeling reaction, 1-2 mg

Kit Contents:

AC025-003-M1:	Link-A-Light Mix (Ready to Use)
AC025-003-R1:	Link-A-Light Modifier reagent (Ready to Use)
AC025-003-Q1:	Link-A-Light Quencher reagent (Ready to Use)

Conjugate: ATTO633

Background: The Link-A-Light conjugation kit allows Fluorescent conjugations to set up in *seconds*, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing a proprietary activated Fluorescent 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 (see Principle).
Label: Atto633 is one of a new generation of Fluorescent labels. It has a strong absorption at 629nm, high fluorescence at 657nm (extinction coefficient 1.3 x10e5 cm-1M-1) and high quantum yield. Atto633 can be used as a substitute dye for DY-633, LightCycler Red 640, Alexa Fluor633, and Cy5.

Applications: Labeling

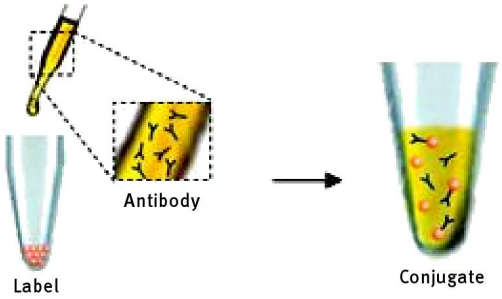


Figure 1

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 coupling of the the antibody to the Fluorescent dye, in a gentle and controlled process at near-neutral pH. Link-A-Light makes it possible to conjugate primary antibodies and other proteins with ease, using a simple, efficient process that has safeguards to prevent over-labeling of the biomolecule and that ensure 100% recovery even at small scale (see Principle).

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 recommended amount of antibody to be used for labeling is 10-20 µg for **AC025-001**, 100-200 µg for **AC025-002** and 1-2 mg for **AC025-003**. The volume of the antibody sample, ideally, should be in the range 4-10 µl for **AC025-001**, 40-100 µl for **AC025-002** and 400-1000 µl for **AC025-003**. Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the suggested ranges have also yielded excellent conjugates. **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:

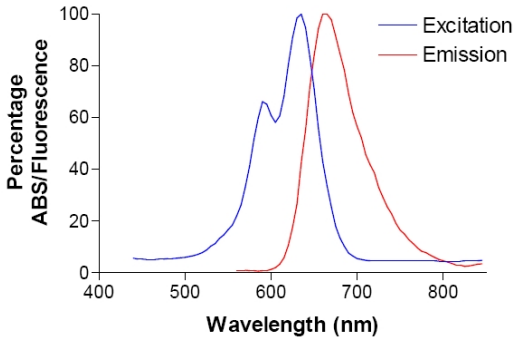
Storage of Conjugates: For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must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

Pictures:

Absorbance Max (nm)	Emission Max (nm)	Excitation Coefficient ($cm^{-1} M^{-1}$)	Fluorescent Colour	Stokes Shift
629	657	130000	Red	28

Excitation and Emission Scan of Link-A-Light ATTO633 (AC025-002 and AC025-003). Scans perormed in TBS, pH8.



License:

Acris Antibodies' Link-A-Light conjugation kits are offered for research purposes alone, and are not intended for human, therapeutic or diagnostic use. The purchase of this conjugation kit conveys to the buyer (whether the buyer is a not-for-profit, academic or for-profit entity) the non-transferable right to use the amount of product purchased and the components of the product for in-house research. The buyer shall not sell or otherwise transfer this product, its components, or materials prepared therefrom to any third party. The buyer shall not use this product or its components for commercial purposes. For the avoidance of doubt, 'commercial purposes' means any activity by a party for consideration and includes, without limitation, use of the product or its components (i) in the manufacturing of conjugated materials (e.g. labeled antibodies), (ii) to provide a service, information or data, (iii) for therapeutic, diagnostic or prophylactic purposes, or (iv) for repackaging/resale, whether or not such product or its components are resold for use in research. The use of this product by the buyer constitutes agreement with the terms of this limited use label license for Link-A-Light products. For further information please contact Acris Antibodies GmbH, Schillerstraße 5, D-32052 Herford, Germany. Tel: +49-5221-34606-0, Fax +49-5221-34606-11, e-mail: info@acris-online.de