

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

Catalog No.:	AC010-002
Quantity:	1 kit
Reaction:	3 labeling reactions, each 100-200 µg
Kit Contents:	AC010-002-M1: Link-A-Light Mix (Ready to Use) AC010-002-R1: Link-A-Light Modifier reagent (Ready to Use) AC010-002-Q1: Link-A-Light Quencher reagent (Ready to Use)
Conjugate:	AMCA
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 AMCA conjugations to set up in seconds, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing AMCA (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).

Unlike standard AMCA labeling procedures, where a large molar excess of AMCA is employed, the Link-A-Light AMCA kit combines a low molar ratio of AMCA:Antibody and high labeling efficiency to eliminate the need for purification steps.

Label: AMCA (aminomethylcoumarin acetate) is a blue Fluorescent dye commonly used in Fluorescence Microscopy, arrays and in-situ hybridisation. It has an Excitation wavelength of 345-350nm and an Emission wavelength of 440-460nm.

Applications: Labeling

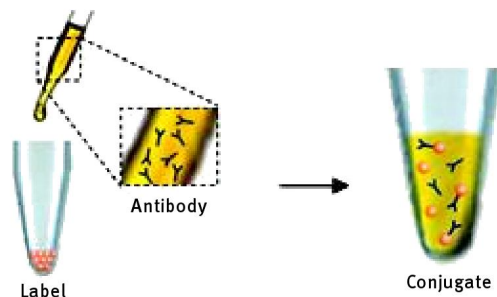


Figure 1

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 Fluorescent 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 PE with ease, and eliminates the need for secondary detection reagents in Immunoassay procedures such as Western Blotting, ELISA and Immunocytochemistry.

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 AC010-001, 100-200 µg for AC010-002 and 1-2 mg for AC010-003. The volume of the antibody sample, ideally, should be in the range 4-10 µl for AC010-001, 40-100 µl for AC010-002 and 400-1000 µl for AC010-003. Antibody concentrations of 1-4 mg/ml generally give optimal results, but concentrations and volumes outside the suggested ranges have also yielded good 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:

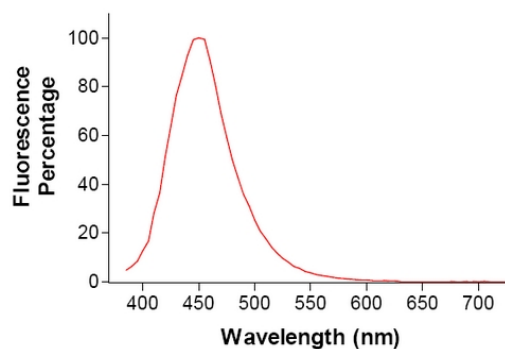
Storage of Conjugates: For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for Long-term storage. Other storage conditions (e.g. Frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must 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)	Extinction Coefficient (cm ⁻¹ M ⁻¹)	Fluorescent Colour	Stokes Shift
353	442	19000	Blue	89

Emission Scan of Link-A-Light AMCA for AC010-002 and AC010-003. Scan performed in TBS, pH 8.0



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