

## Link-A-Light ATTO465 Conjugation Kit (3 labeling reactions, each 10-20 µg)

<b>Catalog No.:</b>	AC019-001
<b>Quantity:</b>	1 kit
<b>Reaction:</b>	3 labeling reactions, each 10-20 µg
<b>Kit Contents:</b>	AC019-001-M1: Link-A-Light Mix (Ready to Use) AC019-001-R1: Link-A-Light Modifier reagent (Ready to Use) AC019-001-Q1: Link-A-Light Quencher reagent (Ready to Use)

**Conjugate:** ATTO465

**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:** Atto465 is a Fluorescent label derived from Acriflavin. It is used to label DNA, RNA or proteins. It has a strong absorption at 453nm, high fluorescence at 508nm (extinction coefficient  $7.5 \times 10^4 \text{ cm}^{-1}\text{M}^{-1}$ ), high quantum yield, and large Stokes-Shift

**Applications:** Labeling

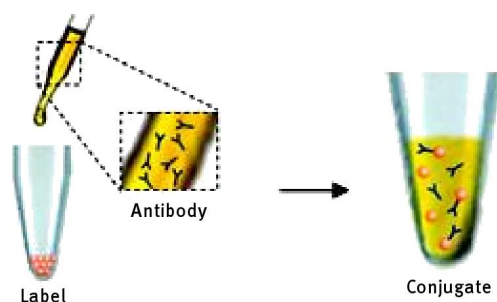


Figure 1

### 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 AC019-001, 100-200 µg for AC019-002 and 1-2 mg for AC019-003. The volume of the antibody sample, ideally, should be in the range 4-10 µl for AC019-001, 40-100 µl for AC019-002 and 400-1000 µl for AC019-003. 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.

**Add. Information:**

Applicable to Western Blotting, ELISA, Immunohistochemistry, Immunofluorescence, and FACS analysis.

**Storage:**

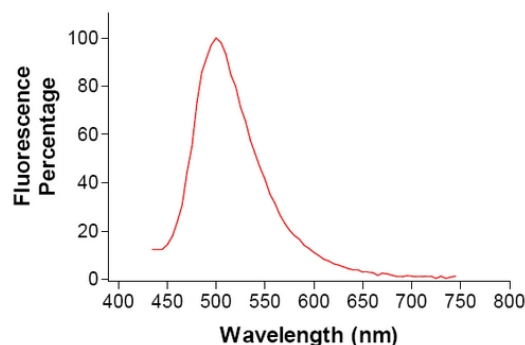
**Storage of Conjugates:** For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate 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 <sup>1</sup> M <sup>-1</sup> )	Fluorescent Colour	Stokes Shift
453	508	75000	Yellow-Green	55

**Emission Scan of Link-A-Light ATTO465 (AC019-002 and AC019-003). Scans performed in TBS, pH8.**



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