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Link-A-Light PE-ATTO594 Conjugation Kit (3 labeling reactions)

Catalog No.: AC040-001

Quantity: 3 x 10 µg

Reaction: 3 labeling reactions

Kit Contents:
AC040-001-M1: Link-A-Light Mix (Ready to Use)
AC040-001-R1: Link-A-Light Modifier reagent (Ready to Use)
AC040-001-Q1: Link-A-Light Quencher reagent (Ready to Use)

Conjugate: PE-ATTO594

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 PE-Atto594 Tandem conjugations to set up in **seconds**, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing PE-Atto594 (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 for FACS analysis with 100% recovery (see Principle).
Label: PE-Atto594 is a Tandem conjugate. The PE is excited at 488nm and functions as an energy donor for the Atto 594 Fluorescent dye. Energy is transferred from the PE to the Atto594 via Fluorescence Resonance Energy Transfer (FRET). The Atto594 emits the energy received from the PE in the form of long wavelength light at 627nm.

Applications: Labeling

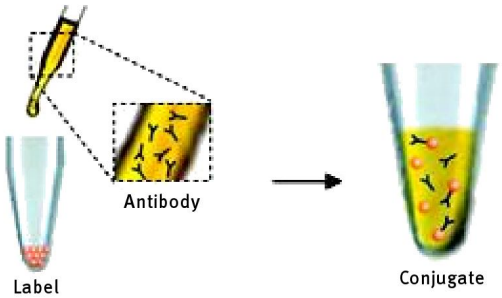


Figure 1

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 with

PE-Atto594 with ease, and eliminates the need for secondary reagents in FACS experiments. Direct labeling can simplify and improve data quality in multicolor experiments by eliminating problems caused by dissociation and crossover of secondary reagents.

Materials Required but Not Provided:

Antibody to be Labeled: In view of the large size of the PE-Atto594 Tandem, the amount of the Tandem is in a slight molar excess. The best ratio for any new antibody reagent must be determined by experimentation but 10 µgof IgG corresponds to AB:PE molar ratio of 1:1. The volume in which the antibody is added ideally should be between 4 µl and 10 µl (10 µg pack size). **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. Glycerol up to 50% has 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:

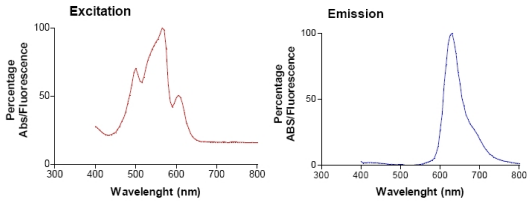
Storage of Conjugates: For any new conjugate, storage at 4°C is recommended. A preservative may be desirable for Long-term storage. Other storage conditions may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

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)	Fluorescent Colour	Stokes Shift
535	627	Red	92

Excitation and Emission Scan of Link-A-Light PE-Atto594. Scan performed in TBS, pH 8.0.



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