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Link-A-Light Rhodamine Conjugation Kit (3 labeling reactions, each up to 20 µg)

Catalog No.: AC009-001

Quantity: 1 kit

Reaction: 3 labeling reactions, each up to 20 µg

Kit Contents:

AC009-001-M1:	Link-A-Light Mix (Ready to Use)
AC009-001-R1:	Link-A-Light Modifier reagent (Ready to Use)
AC009-001-Q1:	Link-A-Light Quencher reagent (Ready to Use)

Conjugate: Rhodamine

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 Rhodamine conjugations to set up in seconds, simply by adding a solution of the antibody to be labeled to a proprietary lyophilised mixture containing Rhodamine (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 Rhodamine labeling procedures, where a large molar excess of rhodamine is employed, the Link-A-Light Rhodamine kit combines a low molar ratio of Rhodamine:Antibody and high labeling efficiency to eliminate the need for purification steps.
Label: Rhodamine dyes are used extensively in biotechnology applications such as Fluorescence Microscopy, Flow Cytometry, Fluorescence correlation spectroscopy and ELISA. Rhodamine has an Emmission wavelength of 576nm and an Excitation wavelength of 544nm.

Applications: Labeling

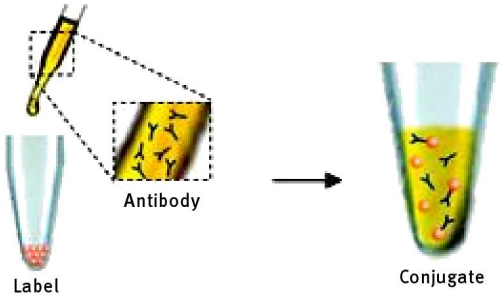


Figure 1

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.

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 Rhodamine. e.g. For 100 µg Link-A-Light Rhodamine add 100 µg of the antibody. The volume in which the antibody is added ideally should be 4-10 µl (10 µg pack size), 40-100 µl (100 µg pack size) and 0.4-1ml (1 mg pack size). Antibody concentrations in the range 1-4 mg/ml are therefore ideal. However, concentrations and volumes outside these suggested limits have also yielded excellent conjugates. For any new antibody, optimization of the ratio of antibody to Rhodamine 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.

Add. Information:

The Rhodamine used in the Link-A-Light kit has an Absorption peak at 520 and 544 nm. Maximum Emission is obtained when the Rhodamine is excited at 544 nm.

Storage:

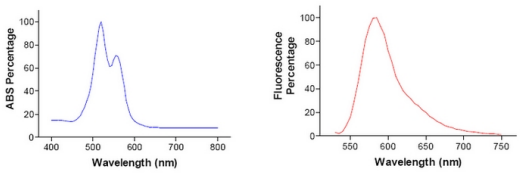
Storage of Conjugates: For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for Long-term storage. Other storage conditions (e.g. Frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must 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
544	576	94500	Orange	32

Excitation (Left) and Emission (Right) Scan of Link-A-Light Rhodamine.



Scan Performed in TBS pH 8.0

License:

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