



Lightning-Link® Conjugation System

Fluorescent dyes: This protocol applies to product codes starting 707, 710, 712, 714, 730-751, 780-793

Version 3

Storage and components

These kits are shipped at ambient temperature in a tamper-evident polypropylene container. Store at -20°C upon receipt.

Kit contents

Glass vial(s) of Lightning-Link® mix
(1 or 3 vials, depending on pack size)

1 vial of LL-Modifier reagent

1 vial of LL-Quencher FD reagent

Pre-conjugation considerations

Antibody buffer

The purified antibody to be labeled should ideally be in 10-50mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5. If the buffer is more concentrated or outside this pH range contact the technical support team.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. Azide (0.02 to 0.1%) and BSA (0.1 to 0.5%) have little or no effect. Glycerol up to 50% has no effect.

Avoid buffer components that are nucleophilic, as these may react with Lightning-Link® chemicals. Primary amines (e.g. amino acids, Tris or ethanolamine) and thiols (e.g. mercaptoethanol or DTT) fall within this class. Thimerosal (thiomersal) should also be avoided.

Recommended amount and volume of antibody for optimal results

Pack size	Amount of antibody	Volume of antibody
Up to 20µg	10-20µg	4-10µl
Up to 200µg	100-200µg	40-100µl
Up to 2mg	1-2mg	400-1000µl

Antibody concentrations of 1-4mg/ml generally give optimal results. For conditions outside the suggested ranges contact the technical support team.

Conjugation reaction

1. **Before** adding the antibody to the Lightning-Link® mix, add 1µl of LL-Modifier reagent to each 10µl of antibody to be labeled. Mix gently.
2. Remove cap from vial of Lightning-Link® mix and pipette the antibody sample (with added LL-Modifier) directly onto the lyophilised material. Resuspend *gently* by withdrawing and re-dispensing the liquid once or twice using a pipette.
3. Replace cap on the vial and leave standing for 3 hours in the dark at room temperature (20-25°C). Conjugations can also be set up and left overnight; longer incubation times have no negative effect on the conjugate.
4. After incubating for 3 hours (or more), add 1µl of LL-Quencher FD reagent for every 10µl of antibody used and mix gently. The conjugate can be used after 30 minutes. The conjugates do not require purification.

Storage of conjugates

Storage at 4°C is recommended for any conjugate. 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.

Technical support team

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