ReadiLink™ Digoxigenin (DIG) Antibody Conjugation Kit *Microscale Optimized for Labeling 100 µg Antibody Per Reaction*

Catalog number: 5455
Unit size: 2 Labelings

Component	Storage	Amount (Cat No. 5455)
Component A: Digoxigenin, SE	Freeze (< -15 °C)	2 vials
Component B: Reaction Buffer	Refrigerated (2-8 °C)	1 vial (20 µL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 µL)
Component D: 30K Spin Filter Set	Room temperature (10-25 °C)	2 filters

OVERVIEW

ReadiLink™ Digoxigenin (DIG) Antibody Conjugation Kit enables efficient and consistent labeling of antibodies with digoxigenin, a widely used hapten in hybridization assays and immunodetection platforms. This kit uses an NHS ester–based chemistry for covalent attachment of DIG to primary amines commonly found on lysine residues of the antibody. It is designed for simple mix-and-incubate protocols, with minimal purification required. Each kit includes all necessary components to label up to 200 µg of antibody (100 µg per reaction × 2 vials). The entire process is completed in ~1 hour. The resulting DIG-labeled antibodies are compatible with anti-DIG detection reagents and suitable for use in ELISA, in situ hybridization (ISH), dot blotting, and related applications. This format provides a safe and reliable alternative to radioactive labeling and is ideal for laboratories seeking rapid DIG conjugation.

AT A GLANCE

Protocol Summary:

1. Add 5 µL DMSO (Component C) to one vial of Digoxigenin, SE (Component A).
2. Add 10 µL Reaction Buffer (Component B) into target antibody (100 µL).
3. Add the antibody solution into reconstituted Digoxigenin, SE
4. Incubate at room temperature for 30-60 minutes.
5. Purify the conjugate by 30K Spin Filter (Component D).

Important Note:

1. Upon receipt, store Digoxigenin, SE (Component A) at -20 °C, kept away from moisture. Do not freeze Spin Column (Component D). Warm all the components before opening, and prepare the required solutions immediately before starting the conjugation. This SOP was developed for Goat anti-Rabbit IgG labeling, and you might need further optimization for your antibody labeling.
2. Warm all the components and centrifuge the vials briefly before opening, and immediately prepare the required solutions.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Digoxigenin, SE stock solution

Add 5 µL of DMSO (Component C) into the vial of Digoxigenin, SE (Component A), and vortex vigorously.

Note: Prepare the Digoxigenin, SE stock solution before starting the conjugation. Use promptly.

PREPARATION OF WORKING SOLUTION

Antibody working solution

For labeling 100 µg of antibody (assuming the target antibody concentration is 1mg/mL), mix 10 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 100 µL of the antibody solution.

Notes:

1. For labeling 100 µg of antibody (assuming the target antibody concentration is 1 mg/mL), mix 10 µL (10% of the total reaction volume) of Reaction Buffer (Component B) with 100 µL of the target antibody solution.

2. The antibody should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2 - 7.4. If the antibody is dissolved in glycine buffer, it must be dialyzed against 1X PBS, pH 7.2 - 7.4, or use Amicon Ultra-0.5, Ultracel-10 Membrane, 30 kDa (cat# UFC501008 from Millipore) to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for antibody precipitation.

3. Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well.

4. For optimal labeling efficiency, a final antibody concentration range of 1 - 2 mg/mL is recommended, with a significantly reduced conjugation efficiency at less than 1 mg/mL.

SAMPLE EXPERIMENTAL PROTOCOL

Setting up the reaction:
Add the antibody working solution to ONE vial of reconstituted Digoxigenin, SE (Component A), and mix them well by repeatedly pipetting for a few times or vortex the vial for a few seconds. Keep the conjugation reaction mixture at room temperature for 30-60 minutes. Note: The conjugation reaction mixture can be rotated or shaken for longer time if desired.

Purification:

Transfer the labeling reaction to the membrane of the 30K Spin Filter (Component D) provided in the kit. Centrifuge the vial at 6,000 x g until all of the liquid has filtered into the receiving vial. Resuspend the labeled antibody in PBS to ~500 µL and centrifuge again. Repeat this cycle for four times, discarding the flow through each time. Collect the solution containing digoxigenin labeled antibody.

Final Conjugate:

Add the storage buffer (of your choice) to the concentration desired, pipette up and down over the upper surface of the membrane to recover and resuspend the antibody. Transfer the recovered antibody solution to a fresh micro centrifuge tube. The antibody conjugate should be stored at > 0.5 mg/mL in the presence of a carrier protein (e.g., 0.1% bovine serum albumin). The conjugate solution could be stored at 4 °C for two months without significant change when stored in the presence of 2 mM sodium azide and kept from light. For longer

storage, the protein conjugates could be lyophilized or divided into single-used aliquotes and stored at ≤ –60 °C.

DISCLAIMER

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