GeneCopoeia™
Expressway to Discovery

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iLink™ Biotin Antibody Labeling Kit
Catalog Number: L020

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
Biotin-X NHS Ester (Component A)	**3 vials**	**-20 °C**	**The product is stable for at least six month when stored as directed.**
Activation Reagent (Component B)	**100 «L**	**-20 °C**	
Quencher Reagent (Component C)	**100 «L**	**-20 °C**	
Storage Buffer (Component D)	**1 mL**	**-20 °C**	
Ultrafitration Vial (MWCO=10K)	**3 vials**	**RT**	

Number of labeling: 3 labeling optimized for 50~100 µg of a monoclonal antibody.

Introduction

iLink™ Biotin Antibody Labeling Kit provides a fast and convenient means to label small amounts of monoclonal antibodies with biotin molecule. Monoclonal antibodies are often available only in small quantities and this kit is optimized for labeling 50~100 µg per reaction. iLink™ Biotin Antibody Labeling Kit contains everything you need to rapidly label an antibody with biotin. The labeling procedure comprises simple mixing of your antibody with a vial of lyophilized mixture containing the label of interest, followed by a brief incubation (Figure 1).

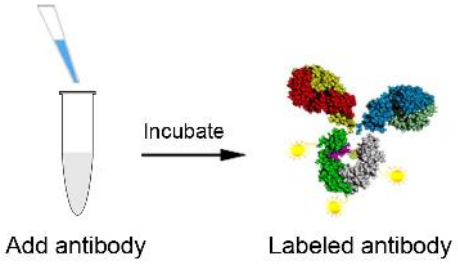

Figure1. Antibody labeling workflow

After labeling, the biotin molecule is covalently linked to the antibody with a degree of labeling of approximately 4-8 biotin molecules per antibody molecule. A microcentrifuge ultrafiltration vial is provided in the kit, which can be used to rapidly remove incompatible small molecule antibody stabilizers before labeling if needed, and remove excess of biotin molecule after labeling.

Our iLink™ Antibody Labeling Kit makes it possible to label primary antibodies and other proteins with ease, and eliminates the need for the secondary reagents in immunoassay procedures such as western blotting, ELISA and immunocytochemistry.

Experimental Protocols

Antibody Concentration or Clean-up (*Optional*)

Important: The antibody need be in a buffer free of ammonium ions or primary amines. If the antibody in or has been lyophilized from an unsuitable buffer (e.g. Tris or glycine) or purified with ammonium sulfate, the

buffer needs to be replaced with PBS buffer by ultrafiltration before labeling. The optimal antibody concentration for labeling is 0.5~1 mg/mL. If the antibody concentration is less than 0.5 mg/mL, concentrate the antibody by ultrafiltration before labeling.

1.1 Add antibody to the ultrafiltration vial, being careful not to touch the membrane. Spin the solution at 14,000 x g in a microcentrifuge for one minute. Check to see how much liquid has filtered into the filtrate collection tube (lower chamber). Repeat the centrifugation until all of the liquid has filtered into the collection tube. Discard the liquid in the collection tube.

1.2 Add 500 µL PBS to the ultrafiltration vial. Spin the vial at 14,000 x g until the liquid has filtered into the collection tube.

1.3 Add an appropriate amount of PBS to the ultrafiltration vial to obtain a final antibody concentration of 0.5 - 1 mg/mL. Carefully pipette the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody.

1.4 Transfer the recovered antibody solution to a new microcentrifuge tube, and save the ultrafiltration vial to concentrate your antibody after labeling.

Antibody Labeling

2.1 Warm up the kit components to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.

2.2 Use 50~100 µg antibody at a concentration of 0.5-1 mg/mL for optimal labeling. If the antibody is in a lyophilized form or is more concentrated, reconstitute or dilute the antibody in PBS. Transfer the antibody to be labeled to a clean tube.

2.3 Mix the **Activation Reagent** (Component B) with the antibody solution at a ratio of 1:9 (For example, mix 10 µL of **Activation Reagent** with 90 µL of antibody solution). Mix the solutions by pipetting up and down a few times.

2.4 Transfer the entire solution from Step 2.3 to one vial containing the **Biotin-X NHS Ester** (Component A). Vortex the vial for a few seconds.

2.5 Incubate the vial in the dark for 30 minutes at room temperature.

2.6 Add one-tenth volume of **Quencher Reagent** (Component C) to above antibody labeling solution. Vortex the vial for a few seconds, and incubate the vial in the dark for 5 minutes at room temperature.

2.7 Transfer the entire solution from Step 2.6 to the ultrafiltration vial saved from step 1.4. Spin the vial at 14,000 x g until the liquid has filtered into the collection tube.

2.8 Resuspend the labeled antibody in **Storage Buffer** (Component D) at desired final concentration. Carefully pipette the storage buffer up and down over the upper surface of the membrane to recover and resuspend the antibody.

2.9 Transfer the recovered antibody solution to a new collection tube. The antibody is now ready to use for staining.

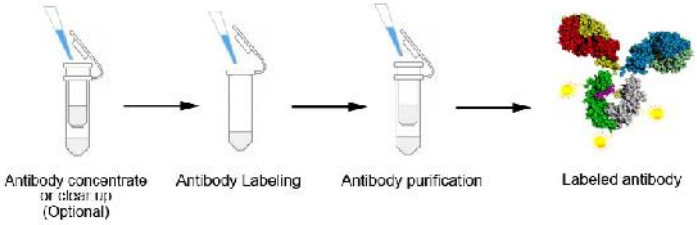

Figure 2. Antibody labeling process