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iLink™ Andy Fluor 568 Antibody Labeling Kit
Catalog Number: L036

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
Andy Fluor 568 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 µL**	**-20 °C**	
Quencher Reagent (Component C)	**100 µL**	**-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.
Fluorescence excitation and emission maxima: 578/602 nm

Introduction

iLink™ Andy Fluor 568 Antibody Labeling Kit provides a fast and convenient means to label small amounts of monoclonal antibodies with Applied BioProbes' next-generation Andy Fluor 568 dye. Monoclonal antibodies are often available only in small quantities and this kit is optimized for labeling 50~100 µg per reaction. iLink™ Andy Fluor 568 Antibody Labeling Kit contains everything you need to rapidly label an antibody with Andy Fluor 568. 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).

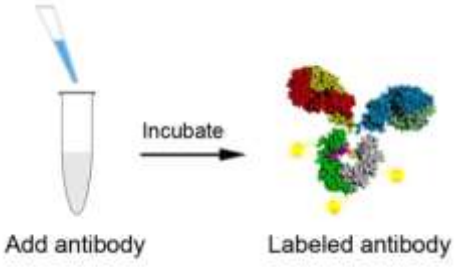

Figure1. Antibody labeling workflow

After labeling, the Andy Fluor 568 dye is covalently linked to the antibody with a degree of labeling of approximately 3-8 dye 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 dye 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 **Andy Fluor 568 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

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Frequently Asked Questions

Question	Answer
Can I use the iLink™ Antibody Labeling Kits for labeling proteins other than antibodies?	iLink™ Antibody Labeling Kits are optimized for labeling IgG antibodies. We do not recommend them for labeling other proteins. iLink™ labeling conditions for other proteins need be optimized by customer.
Is the labeled antibody free of any unconjugated dye or biotin molecule?	Yes. The last ultrafiltration step removes the unconjugated free dye or biotin molecule.
What dye/protein ratio should I use to ensure optimal labeling?	The iLink™ Antibody Labeling Kits have been optimized to label 50~100 µg of antibody. There is no need to measure the dye amount.
Is staining with iLink™ labeled antibodies as sensitive as staining with unlabeled primary and fluorescent secondary antibodies?	Direct immunofluorescence detection can be less sensitive than indirect detection.
Can I use iLink™ labeled antibodies for multi-color immunofluorescence staining, or will the dye transfer between antibodies?	iLink™ labeling results in covalent linkage of dye and antibody, so there will be no dye diffusion or transfer.
How important is the antibody concentration?	The kits are optimized for labeling antibodies with a concentration between 0.5-1.0 mg/mL. If your antibody solution is too dilute, you can concentrate it by centrifugation using the ultrafiltration vial provided in the kit. If your antibody solution is too concentrated, you can dilute it with 1x PBS. Antibody concentrations outside the recommended range may result in either under- or over-labeling.
Can I split the kit contents and use it more than one time?	No. Each vial of dye component in the iLink™ kits is optimized for one labeling. We do not recommend that you try to split the kit to label more than one antibody or for more than one use.
What are the advantages of using directly labeled conjugates compared to indirect staining with labeled secondary antibodies?	Direct immunofluorescence staining eliminates the need for secondary antibody incubation and wash steps, and allows the use of multiple primary antibodies from the same species for multicolor detection, or staining of animal tissues with antibodies raised in the same species without secondary antibody cross-reactivity.