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Mix-n-Stain™ Labeling Kit FAQ

For CF™ Dye Antibody Labeling Kits

How do I remove any unconjugated free dye from the labeled antibody since there is no purification step?

This question relates to a key element of our invention. The unique formulations of our dyes and buffers and the labeling strategy have completely removed this concern, which normally has to be dealt with when using conventional antibody labeling methodology. The exact mechanism on how this problem is solved is proprietary information.

Can I use Mix-n-Stain™ labeled antibodies for multi-color immunofluorescence staining, or will the dye transfer between antibodies?

Mix-n-Stain™ labeling results in covalent linkage of dye and antibody, so there will be no dye diffusion or transfer. Please refer to the Introduction section on page 1 for more details.

Can I use a Mix-n-Stain™ kit for labeling proteins other than antibodies?

Mix-n-Stain™ kits are optimized for labeling IgG antibodies. We do not recommend them for labeling other proteins. Mix-n-Stain™ labeling conditions may cause denaturation of IgM antibodies.

Is staining with Mix-n-Stain™ labeled antibodies as sensitive as staining with unlabeled primary and fluorescent secondary antibodies?

Direct immunofluorescence detection can be less sensitive than indirect detection. Please refer to the Introduction section on page 1 for more details.

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 (e.g. mouse-on-mouse staining).

What are the advantages of Mix-n-Stain™ kits over Invitrogen's Zenon® antibody labeling kits?

The major advantages are: 1) the CF dye is covalently attached to the antibody to eliminate dye transfer or diffusion between antibodies during multi-color staining; 2) Mix-n-Stain™ conjugates are stable for several months in storage buffer whereas Zenon labeling reagents are required to

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be used within 30 minutes; 3) Mix-n-Stain™ conjugates are less bulky because the dyes are directly linked to the antibody, unlike Zenon conjugates which use antibody fragments; 4) No dye/protein optimization is needed, just mix and then stain; 5) No post-staining fixation is required with Mix-n-Stain™; 6) Unlike Zenon, Mix-n-Stain™ labeling is not species-specific.

What are the advantages of Mix-n-Stain™ kits over Innova Bioscience's LightningLink™ Rapid antibody labeling kits?

Mix-n-Stain™ antibody labeling kits use novel CF dyes which are brighter and more photostable than the dyes provided in Lightning Link kits. Mix-n-Stain™ kits are sized for labeling smaller amounts of antibody and are sold as a single labeling, providing more flexibility compared to Lightning Link kits.

What are CF dyes?

CF dyes are highly water soluble, small organic dyes designed for labeling proteins and nucleic acids. With a series of more than 20 colors, many of our CF dyes are brighter and more photostable than competing dyes.

How do I select a Mix-n-Stain™ kit?

For each CF dye, there are three labeling kits for labeling of antibody quantities in three different ranges: 1) 5-20 µg, 2) 20-50 µg, and 3) 50-100 µg. For antibody labeling in the absence of stabilizer protein, select a kit that matches with the amount of your antibody. For antibody labeling in the presence of stabilizer protein or ascites fluid, see Table 2 of the product protocol.

If my antibody amount falls between two kits, which one should I use? For example, if I want to label 50 µg of antibody, should I purchase the 50-100 µg kit or the 20-50 µg kit?

Although either kit will produce good results, it is better to use the smaller kit size if your antibody amount falls between two kit sizes.

What dye/protein ratio should I use to ensure optimal labeling?

There is no need to measure the dye amount or vary the reaction time as long as the amount of your antibody to be labeled falls within the range specified for each kit. With Mix-n-Stain™ labeling kits optimal labeling is ensured because of the proprietary dyes and reaction buffer.

Can I split the kit contents and use it more than one time?

No. The Mix-n-Stain™ kits are optimized for 1 labeling. We do not recommend that you try to split the kit to label more than one antibody or for more than one use.

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

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dilute it with 1x PBS. Antibody concentrations outside the recommended range may result in either under-labeling or over-labeling.

I performed immunofluorescence staining with my labeled antibody, but I don't see any signal. What should I do?

Check with the antibody manufacturer to confirm that the antibody formulation and concentration are compatible with the kit labeling protocol you selected.

You should confirm that your primary antibody is sensitive and specific for your application using indirect labeling before attempting direct labeling. You may need to use a higher concentration of primary antibody to achieve similar signal intensity with direct labeling as with indirect labeling. Please refer to the Introduction section on page 1 for more information.

Covalent labeling may affect the reactivity of certain antibodies. You can test if this is the case by performing indirect immunofluorescence labeling with your Mix-n-Stain™ labeled primary with secondary detection using a fluorescently-labeled secondary antibody to confirm that the primary antibody is still reactive.

If you have access to a fluorescence gel reader or scanner that is compatible with the excitation/emission wavelengths of the dye you are using, you can confirm labeling of your antibody by performing denaturing SDS-PAGE on a small amount (0.1-0.5 µg) of labeled antibody, then imaging the gel fluorescence. You should be able to detect fluorescent bands representing IgG heavy and light chains at ~55 kDa and ~25 kDa.

Other Mix-n-Stain™ Antibody Labeling Kits

GoldBio Catalog #	Product Name
A-825	Mix-n-Stain™ AP Antibody Labeling Kit
B-825	Mix-n-Stain™ Biotin Antibody Labeling Kit
F-825	Mix-n-Stain™ FITC Antibody Labeling Kit
G-825	Mix-n-Stain™ Glucose Oxidase (GOX) Antibody Labeling Kit
H-825	Mix-n-Stain™ HRP Antibody Labeling Kit

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Reference Guide to Ordering GoldBio CF™ dyes

Label/Dye	Ex (nm)	Em (nm)	GoldBio Catalog #
CF™350	347	448	CF-350
CF™405S	404	431	CF-405L
CF™405M	408	452	CF-405M
CF™405L	395	545	CF-405S
CF™440	440	515	CF-440
CF™488A	490	515	CF-488A
CF™514	516	548	CF-514
CF™532	527	558	CF-532
CF™543	541	560	CF-543
CF™555	555	565	CF-555
CF™568	562	583	CF-568
CF™594	593	614	CF-594
CF™633	630	650	CF-633
CF™640R	642	662	CF-640R
CF™647	650	665	CF-647
CF™660C	667	685	CF-660C
CF™660R	663	682	CF-660R
CF™680	681	698	CF-680
CF™680R	680	701	CF-680R
CF™750	755	777	CF-750
CF™770	770	797	CF-770
CF™790	784	806	CF-790