Biotium
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Mix-n-Stain™ Frequently Asked Questions and Kit Selection Guide

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Table 1. Mix-n-Stain™ Frequently Asked Questions

Question	Answer
What are CF dyes?	CF dyes are highly water soluble, small organic dyes designed by scientists at Biotium for labeling proteins and nucleic acids. With a series of close to 20 colors, many of our CF dyes are brighter and more photostable than competing dyes. For more information, please see the product flyers for individual CF dyes and the CF dye brochure and FAQ at www.biotium.com.
How do I select a Mix-n-Stain kit?	See the Mix-n-Stain Kit Selection Guide, Table 2, below.
What dye/protein ratio should I use to ensure optimal labeling with Mix-n-Stain?	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.
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 ultra-filtration 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.
Is the dye covalently attached to lysine or cysteine side chain in the antibody?	The dye is covalently attached to the side chains of amino acids located far away from the antigen-binding sites so that the affinity of the antibody is not affected. However, the exact attachment sites and the nature of chemical linkages are proprietary information.
How many dye molecules will be attached to my antibody after labeling?	The degree of labeling with Mix-n-Stain kits is estimated to be in the range of 4-6 dye molecules per antibody.
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.
What is the recovery yield?	~100%.
How long does it take to finish the whole process?	It takes 30 seconds or less to mix the components (antibody, dye and reaction buffer). After another 30 minutes of hands-free reaction time, you are done! You can use the antibody right away to stain your sample for microscopy, flow cytometry, Western analysis or other applications requiring fluorescently labeled antibodies.
Can I let the reaction go over 30 minutes?	The labeling reaction takes only 30 min. However, longer reaction time will not adversely affect the labeling.
Can I shorten the labeling reaction time to less than 30 min?	The optimal reaction time is 30 minutes. However, shortening the reaction to 20 minutes still produces good results. If the reaction time is less than 30 min, you must combine the labeled antibody solution with the storage buffer provided before you use it to stain your sample.
How stable is the labeled antibody?	Your labeled antibody is stable for at least six months if stored in the Mix-n-Stain storage buffer at 4°C . For longer-term storage, you can aliquot your antibody and store at -20°C.
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.
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. You may need to use a higher concentration of antibody or higher gain settings to achieve similar staining intensity compared to indirect immunofluorescence staining. In our internal testing, indirect immunofluorescence staining results in about 3-fold signal amplification compared to direct immunofluorescence staining.
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?	1) Unlike Zenon, Mix-n-Stain labeling covalently attaches the dye to the antibody, eliminating dye transfer or diffusion between antibodies during multi-color staining; 2) Mix-n-Stain conjugates are stable for at least 6 months in storage buffer, whereas Zenon complexes must 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 post-staining fixation is required with Mix-n-Stain; 5) Unlike Zenon, Mix-n-Stain labeling is not species-specific.
What are the advantages of Mix-n-Stain kits over Innova Bioscience's Lightning-Link™ 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 single labelings, providing more flexibility compared to Lightning Link kits.
Can I use a Mix-n-Stain kit for labeling proteins other than antibodies?	Mix-n-Stain kits are optimized for labeling IgG antibodies. Customers have reported successful labeling of nanobodies and single chain antibodies. Mix-n-Stain kits labeling conditions may cause denaturation of IgM antibodies. We do not have data on labeling of non-antibody proteins using the kits.

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 trying to split the kit to label more than one antibody or for more than one use.
What buffers are compatible with the labeling?	PBS, HEPES, MES, MOPS or borate buffers are compatible. Buffers containing up to 20 mM Tris are also compatible. Tris at levels higher than 20 mM should be removed using the ultrafiltration vial provided in the kit. See Table 2 below for more information.
Is the labeling reaction compatible with small molecule stabilizer/buffer components, such as sodium azide, EDTA, sugars, glycerol, DTT, 2-mercaptoethanol, glycine, and amino acids?	Sodium azide, EDTA, small sugars, and >10% glycerol have no effect on the labeling. Higher levels of glycerol, or any level of DTT, 2-mercaptoethanol or free amino acids (such as glycine) should be removed using the ultrafiltration vial provided in the kit. See Table 2 below for more information.
Can the labeling reaction tolerate the presence of BSA or gelatin?	Labeling IgG free of BSA or gelatin stabilizer gives the best results. However, IgG containing BSA or gelatin can be labeled with good results using a larger sized kit and our modified Mix-n-Stain protocol. See Table 2 below to select the appropriate kit size and protocol.
Is the labeling reaction compatible with ascites fluid, serum or hybridoma supernatant?	IgG in ascites fluid can be labeled with good results using a larger sized kit and our modified Mix-n-Stain protocol. See Table 2 below to select the appropriate kit size and protocol. Mix-n-Stain labeling is does not work well for antibodies in serum or hybridoma cell culture supernatant. We recommend purifying IgG before labeling. See Table 2 for more information.
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. Covalent labeling may affect the reactivity of certain antibodies. You can confirm that the labeled antibody is still reactive by performing indirect immunofluorescence labeling with your Mix-n-Stain labeled primary followed by a fluorescently-labeled secondary antibody. You can confirm labeling of your antibody by performing denaturing SDS-PAGE on a small amount (0.1-0.5 ug) of labeled antibody, then imaging the gel fluorescence at the appropriate excitation/emission wavelengths of the CF dye you used. You should be able to detect fluorescent bands representing IgG heavy and light chains at ~55 kDa and ~25 kDa.

Table 2. Mix-n-Stain Kit Selection Guide

Antibody formulation	Kit and protocol selection	Notes/Examples
Purified IgG containing: • Sodium azide • Less than 20 mM Tris • Less than 10% glycerol	1. Choose the kit size based on the amount of IgG you wish to label 2. Use the standard labeling protocol	If the amount of antibody you wish to label falls between two kits sizes, we recommend using the smaller kit size. For example, if you wish to label 20 ug IgG, choose the 5-20 ug-sized kit.
Purified IgG containing: • More than 20 mM Tris • More than 10% glycerol • Glycine • Less than 0.5 ug/uL IgG	1. Choose the kit size based on the amount of IgG you wish to label 2. Perform ultrafiltration using the spin vial provided in the kit 3. Use the standard labeling protocol	If the amount of antibody you wish to label falls between two kits sizes, we recommend using the smaller kit size. For example, if you wish to label 20 ug IgG, choose the 5-20 ug-sized kit.
Purified IgG containing: • Less than 4:1 BSA:IgG by weight • Less than 4:1 gelatin:IgG by weight	1. Choose the kit size based on the amount of IgG you wish to label 2. Use the standard labeling protocol	For example, if you wish to label 5 ug IgG in 5 uL PBS containing 0.1% BSA. The BSA:IgG ratio by weight is 5 ug BSA:5 ug IgG or 1:1. Select a 5-20 ug-sized kit and follow the standard protocol.
Purified IgG containing: • More than 4:1 BSA:IgG by weight • More than 4:1 gelatin:IgG by weight	1. Choose the kit size based on the total amount of protein (IgG + BSA/gelatin) in the volume of antibody solution you wish to label 2. Use the modified labeling protocol	For example, if you wish to label 5 ug IgG in 5 uL PBS containing 1% BSA. The BSA:IgG ratio by weight is 50 ug BSA: 5 ug IgG or 10/1. Select a 50-100 ug-sized kit based on 55 ug of total protein in the labeling reaction and follow the modified labeling protocol. If the total protein amount falls between two kit sizes, you may get better results with the larger kit size.
IgG in ascites fluid	1. Determine the concentration of protein in the ascites fluid 2. Choose the kit size based on the total amount of protein in the volume of ascites fluid you wish to label 3. Use the modified labeling protocol	For example, if you wish to label 10 uL ascites fluid containing 70 ug total protein. Select a 50-100 ug-sized kit based on 70 ug of total protein, and follow the modified labeling protocol.
Less than 5 ug purified IgG	1. Add stabilizer protein such as BSA to the antibody to bring the total amount of protein 5 ug 2. Select a 5-20 ug kit and use the standard labeling protocol	For example, if you wish to label 1 ug IgG sample. Select a 5-20 ug-sized kit. Before labeling, add 4 ug BSA to 1 ug IgG and follow the standard labeling protocol.
IgG in: • Serum • Hybridoma cell culture supernatant	Not compatible due to the low concentration of IgG compared to other proteins in these formats; purify IgG before labeling	Use protein A/G or a commercially available IgG clean-up kit to purify IgG. Determine the concentration of IgG, then select the appropriate kit/protocol based on the amount of IgG you wish to label and the buffer formulation after purification.