Product Information

Mix-n-Stain™ Antibody Labeling Kits

Size: 1 labeling reaction per kit

Storage: -20°C

Stability: Stable for at least 1 year from date of receipt when stored as recommended.

Components:

Component	5-20 ug labeling	20-50 ug labeling	50-100 ug labeling
Dye/label vial	1 vial Component A	1 vial Component A	1 vial Component A
Mix-n-Stain™ Reaction Buffer, 10X	1 vial of 15 uL 99951-1	1 vial of 15 uL 99951-1	1 vial of 30 uL 99951
Mix-n-Stain™ Antibody Storage Buffer	1 vial of 60 uL 99952-2	1 vial of 150 uL 99952-1	1 vial of 300 uL 99952
Ultrafiltration vial (MWCO=10K)	1 each 99956	1 each 99956	1 each 99956

Product Application

Mix-n-Stain™ antibody labeling kits contain everything you need to rapidly label an antibody with Biotium's next-generation CF® dyes, other fluorescent dyes, biotin, digoxigenin, or DNP. The labeling procedure comprises simple mixing of your antibody with the reaction buffer and optimally formulated dye provided, followed by a brief incubation. Any free dye or other label is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. After the reaction, the antibody will be labeled with an average of 4-6 dye/label molecules per antibody molecule. Mix-n-Stain™ labeling is covalent, so labeled antibodies can be used for multiplex staining without transfer of dyes/labels between antibodies.

We also offer Mix-n-Stain™ antibody labeling kits for labeling antibodies with enzymes or fluorescent proteins (see Related Products), and kits for labeling small ligands with dyes.

Kit Compatibility and Protocol Selection

Mix-n-Stain™ antibody labeling kits are optimized for labeling IgG antibodies. The labeling conditions may cause IgM antibodies to denature.

Mix-n-Stain™ labeling can tolerate sodium azide and sugars, as well as low levels of glycerol and Tris. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecule buffer components before labeling, if necessary (see Protocol A).

The standard Mix-n-Stain™ labeling protocol can be performed in the presence of up to four-fold excess of BSA or gelatin to IgG (by ug amount). Simply choose the kit size that corresponds to the amount of IgG you wish to label and follow Protocol B.

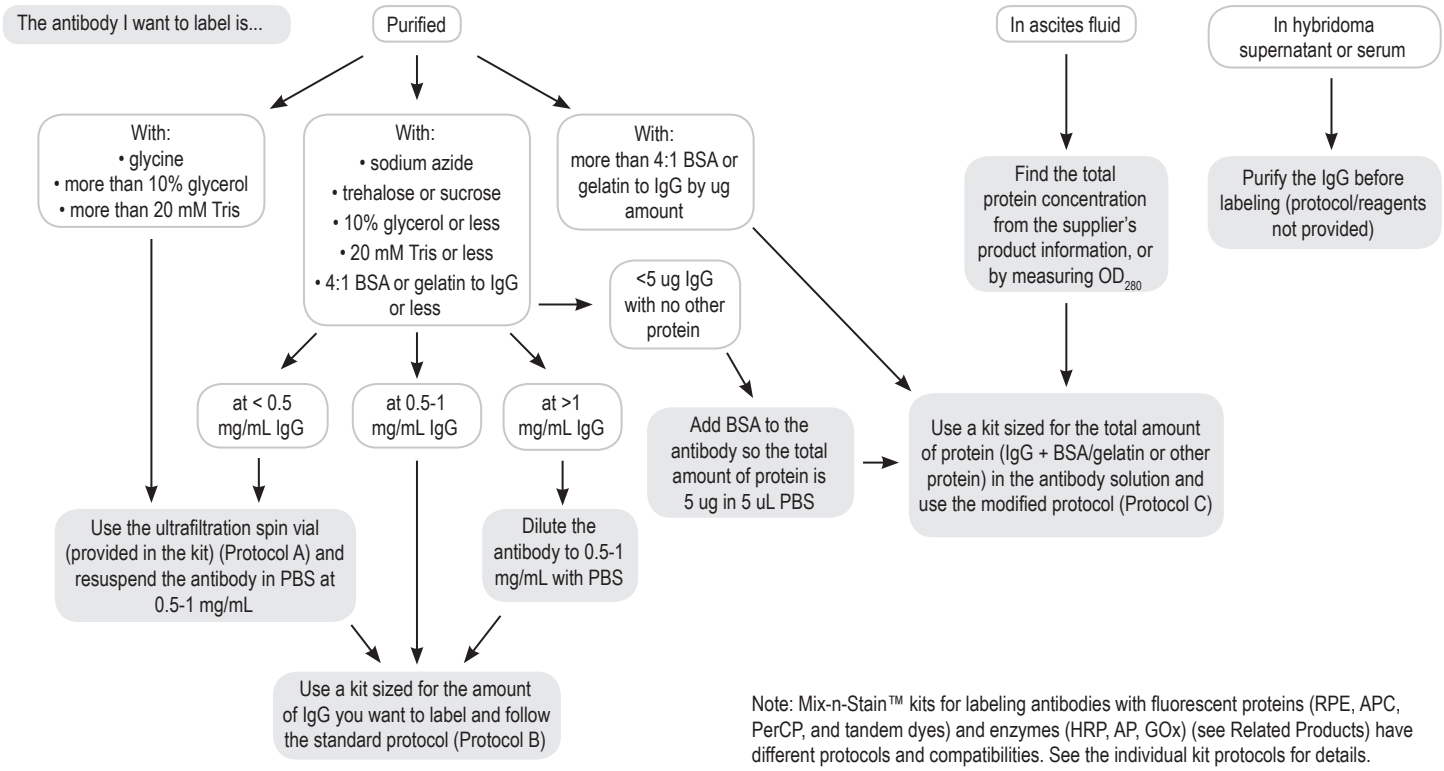
For antibodies that contain more than 4-fold excess BSA or gelatin, we provide a modified Mix-n-Stain™ labeling protocol (Protocol C) that is based on the total amount of protein in the reaction rather than the amount of IgG. Antibodies in ascites fluid also can be labeled using the modified protocol, however you must determine the concentration of total protein in the ascites fluid before labeling. Select the kit size that is appropriate for the total ug amount of protein in the antibody sample that you wish to label.

The modified protocol (Protocol C) also can be used to label antibody amounts that fall below the lower limit of the kit range by adding additional protein to the IgG to bring the total protein amount within the kit range. The modified protocol is not recommended for labeling antibodies in crude antiserum or hybridoma cell culture supernatant due to the low concentration of antibody relative to total protein in these formats.

The optimal antibody concentration for labeling is 0.5-1 mg/mL. The ultrafiltration vial can be used to concentrate antibody solutions by following the steps in Protocol A (note: stabilizer proteins will also be concentrated by the ultrafiltration vial). For quantitating antibodies of unknown concentration, Biotium offers the AccuOrange™ Protein Quantitation Kit, a highly sensitive fluorescence-based protein assay.

See Figure 1 and Table 1 for kit compatibility and protocol selection guidelines. See page 4 for frequently asked questions (FAQs) and troubleshooting tips.

Figure 1. Mix-n-Stain™ Compatibility & Protocol Selection Guide



Note: Mix-n-Stain™ kits for labeling antibodies with fluorescent proteins (RPE, APC, PerCP, and tandem dyes) and enzymes (HRP, AP, GOx) (see Related Products) have different protocols and compatibilities. See the individual kit protocols for details.

Table 1. Mix-n-Stain™ Compatibility and Labeling Protocol Selection Summary

Component	Compatibility
Sodium Azide	Compatible
Glycerol	> 10%: perform ultrafiltration (Protocol A) ≤ 10%: proceed to standard protocol (Protocol B)
Tris	> 20 mM: perform ultrafiltration (Protocol A) ≤ 20 mM: proceed to standard protocol (Protocol B)
Glycine	Perform ultrafiltration (Protocol A)
BSA or gelatin	≤ 4X IgG by ug amount: use standard protocol (Protocol B) > 4X IgG by ug amount: use modified protocol (Protocol C)
Ascites fluid	Use modified protocol (Protocol C)
Serum	Not compatible; purify IgG (protocol/reagents not provided)
Hybridoma cell culture supernatant	Not compatible; purify IgG (protocol/reagents not provided)

A. Ultrafiltration Protocol

Important: Before you begin, see Figure 1 or Table 1 to determine whether your antibody requires ultrafiltration before labeling. If necessary, contact the manufacturer of your antibody to find out the concentration of IgG and antibody stabilizers. If your antibody does not require ultrafiltration, proceed to the appropriate labeling protocol indicated in Fig. 1/Table 1.

The ultrafiltration column membrane has a molecular weight cut-off of 10,000. Therefore, molecules smaller than 10 kDa will flow through the membrane, and molecules larger than 10 kDa, including IgG antibodies, will be retained on the upper surface of the membrane (Figure 2). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

Ultrafiltration Vial Capacities
Maximum Sample Volume: 500 uL
Final Concentrate Volume: 15 uL
Filtrate Receiver Volume: 500 uL
Hold-up Volume (Membrane/Support): < 5 uL

1. Add an appropriate amount of antibody to the membrane of 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.
2. For antibody concentration, proceed to Step 3. For clean-up, add an equal volume of 1X PBS to the membrane. Spin the vial at 14,000 x g until the liquid has filtered into the filtrate receiving tube.
3. Add an appropriate concentration of PBS to the membrane to obtain a final antibody concentration of 0.5 - 1 mg/mL. Carefully pipet the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody.
4. Transfer the recovered antibody solution to a fresh microcentrifuge tube.
5. If you are using the modified antibody labeling protocol, save the ultrafiltration vial to concentrate your antibody after labeling. Additional ultrafiltration vials also can be purchased separately (cat. no. 22004).

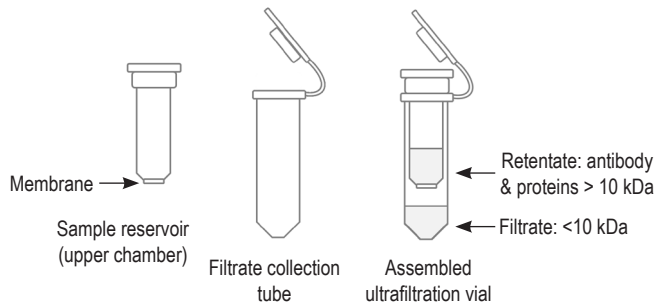


Figure 2. Ultrafiltration vial components.

B. Standard Mix-n-Stain™ Labeling Protocol

Important: Before you begin, use Figure 1 or Table 1 to determine whether your antibody formulation and concentration are compatible with Mix-n-Stain™ labeling, and which labeling protocol you should use.

1. Use your 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. Make sure the ug amount of IgG in the labeling reaction falls within the range of the kit.
2. Warm up the Mix-n-Stain™ Reaction Buffer vial and the Mix-n-Stain™ Storage Buffer vial to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
3. Mix the 10X Mix-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1X Reaction Buffer (for example, mix 9 uL of antibody with 1 uL of 10X reaction buffer). Mix the solutions by pipetting up and down a few times.
4. Transfer the entire solution from Step 3 to the vial containing the dye/label (component A). There is no need to measure the amount of the dye/label in the vial. Vortex the vial for a few seconds.
5. Incubate the vial in the dark for 30 minutes at room temperature.
6. Dilute the labeled antibody solution with the provided Storage Buffer. Simply transfer the entire labeled antibody solution into the Storage Buffer vial and mix. The antibody is now ready to use for staining. The concentration of the labeled antibody is the amount of your starting antibody divided by the total volume.

   Note: Antibody Storage Buffer contains 2 mM sodium azide.

C. Modified Mix-n-Stain™ Labeling Protocol

Important: Before you begin, use Figure 1 or Table 1 to determine whether your antibody formulation and concentration are compatible with Mix-n-Stain™ labeling, and which labeling protocol you should use.

1. Use your antibody solution at a concentration of 0.5-1 mg/mL total protein (IgG plus stabilizer protein) for optimal labeling, using PBS to dilute the solution if necessary. Make sure the ug amount of total protein in the labeling reaction falls within the range of the kit. If you wish to label an amount of IgG that falls below the lower limit of the kit, add BSA to bring to the total protein concentration (IgG + BSA) within the range of the kit and proceed with labeling based on total protein.
2. Warm up the Mix-n-Stain™ Reaction Buffer vial and the Mix-n-Stain™ Storage Buffer vial to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
3. Mix the 10X Mix-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1X Reaction Buffer (for example, mix 9 uL of antibody with 1 uL of 10X reaction buffer). Mix the solutions by pipetting up and down a few times.
4. Transfer the entire solution from Step 3 to the vial containing the dye/label (component A). There is no need to measure the amount of the dye/label in the vial. Vortex the vial for a few seconds.
5. Incubate the vial in the dark for 30 minutes at room temperature.
6. Optional: you can transfer the entire labeling reaction to the tube of antibody storage buffer provided. However, this may result in a highly dilute IgG solution, which may not be practical for subsequent use. To concentrate the antibody before adding to storage buffer, follow the steps below.
7. Transfer the labeling reaction to the membrane of the ultrafiltration vial provided (or saved after antibody clean-up, above). Centrifuge the vial at 14,000 x g until all of the liquid has filtered into the receiving vial as described in Protocol A.

   Note: Ultrafiltration vials also can be purchased separately (see Related Products).
8. Resuspend the labeled antibody in Storage Buffer at the desired final concentration of IgG. Carefully pipette the storage buffer up and down over the upper surface of the membrane to recover and resuspend the antibody.

   Note: Antibody Storage Buffer contains 2 mM sodium azide.
9. Transfer the recovered antibody solution to a fresh microcentrifuge tube. The antibody is now ready to use for staining.

Storage of Labeled Antibodies

Labeled antibodies are stable for at least 6 months when stored at 4°C, protected from light. Antibodies also can be stored in single use aliquots at -20°C for longer term storage.

Considerations for Staining with Mix-n-Stain™ Labeled Antibodies

When performing direct immunofluorescence with a fluorescently-labeled antibody, you may need to use a higher concentration of antibody to achieve similar staining intensity compared to indirect immunofluorescence detection using unlabeled primary plus labeled secondary antibody. In our internal testing, indirect immunofluorescence staining results in about 3-fold signal amplification compared to direct immunofluorescence staining.

Direct labeling should be used with high affinity antibodies against abundant targets. We recommend validating antibodies with secondary detection before attempting direct labeling. Tissue staining with directly labeled fluorescent antibodies can be challenging due to tissue autofluorescence and target integrity issues in human tissue. See our TrueBlack® line of background reducers (Related Products) for reducing background with Mix-n-Stain™ labeled antibodies or labeled secondary antibodies in tissue sections and other samples. We also offer CF® Dye Tyramide Signal Amplification Kits, which can overcome background by amplifying signal, and can be used for multiplexing.

Labeled secondary antibodies will still bind to primary antibodies labeled using Mix-n-Stain™ kits, therefore if multiple primary antibodies from the same species are to be used for multicolor immunofluorescence staining, a secondary antibody cannot be used to distinguish an unlabeled primary antibody from a Mix-n-Stain™ labeled primary antibody from the same species. Mix-n-Stain™ labeled antibodies can be used as a tertiary staining antibody after standard immunofluorescence staining with primary and secondary antibodies. Visit our website to see our Tech Tip: Combining Direct and Indirect Immunofluorescence Using Primary Antibodies from the Same Host.

For more information and troubleshooting tips, see Frequently Asked Questions (page 4).

Ordering information

Label/Dye	Ex/Em (nm)	Labeling size/Catalog number		
		5-20 ug	20-50 ug	50-100 ug
CF®350	347/448	92270	92250	92230
CF®405S	404/431	92271	92251	92231
CF®405M	408/452	92272	92252	92232
CF®405L	395/545	92303	92304	92305
CF®430	426/498	92316	92317	92318
CF®440	440/515	92319	92320	92321
CF®450	450/538	92322	92323	92324
CF®488A	490/515	92273	92253	92233
CF®514	516/548	92331	92332	92333
CF®532	527/558	92289	92290	92291
CF®543	541/560	92287	92267	92247
CF®555	555/565	92274	92254	92234
CF®568	562/583	92275	92255	92235
CF®570	568/591	92334	92335	92336
CF®583	583/606	92337	92338	92339
CF®594	593/614	92276	92256	92236
CF®633	630/650	92277	92257	92237
CF®640R	642/662	92278	92258	92245
CF®647	650/665	92279	92259	92238
CF®660C	667/685	92280	92260	92239
CF®660R	663/682	92281	92261	92243
CF®680	681/698	92282	92262	92240
CF®680R	680/701	92283	92263	92246
CF®750	755/777	92284	92264	92241
CF®770	770/797	92285	92265	92242
CF®790	784/806	92288	92268	92248
Biotin	N/A	92286	92266	92244
DNP	N/A	92325	92326	92327
DIG	N/A	92328	92329	92330
FITC	494/518	92294	92295	92296
Cyanine 555	555/565	92412	92413	92414
Cyanine 647	650/665	92416	92417	92418

Related Products

Catalog number	Product
22004	Ultrafiltration vial, 10K MWCO (pack of 5)
22018	Ultrafiltration vial, 10K MWCO (pack of 5)
30071	AccuOrange™ Protein Quantitation Kit
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
40083	NucSpot® 470 Green Nuclear Counterstain
40061	RedDot™2 Far Red Nuclear Counterstain
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
23006	Flow Cytometry Fixation/Permeabilization Kit
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22020	10X Phosphate Buffered Saline (PBS) (4L Cubitainer®)

Other Mix-n-Stain™ Antibody Labeling Kits

Catalog number	Product
92298	Mix-n-Stain™ R-PE Antibody Labeling Kit, 1 X (25-50 ug) labeling
92299	Mix-n-Stain™ R-PE Antibody Labeling Kit, 1 X (50-100 ug) labeling
92306	Mix-n-Stain™ APC Antibody Labeling Kit, 1 X (25-50 ug) labeling
92307	Mix-n-Stain™ APC Antibody Labeling Kit, 1 X (50-100 ug) labeling
92340	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (25-50 ug) labeling
92341	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (50-100 ug) labeling
92346	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (1 mg) labeling
92310	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kit, 1 X (25-50 ug) labeling
92311	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kit, 1 X (50-100 ug) labeling
92300	Mix-n-Stain™ HRP Antibody Labeling Kit, 1 X (10-20 ug) labeling
92301	Mix-n-Stain™ HRP Antibody Labeling Kit, 1 X (25-50 ug) labeling
92302	Mix-n-Stain™ HRP Antibody Labeling Kit, 1 X (50-100 ug) labeling
92314	Mix-n-Stain™ Alkaline Phosphatase Antibody Labeling Kit, 1 X (25-50 ug) labeling
92315	Mix-n-Stain™ Alkaline Phosphatase Antibody Labeling Kit, 1 X (50-100 ug) labeling
92312	Mix-n-Stain™ Glucose Oxidase Antibody Labeling Kit, 1 X (25-50 ug) labeling
92313	Mix-n-Stain™ Glucose Oxidase Antibody Labeling Kit, 1 X (50-100 ug) labeling

Please visit www.biotium.com to view our full selection of products featuring bright and photostable CF® dyes, including Mix-n-Stain™ Small Ligand Labeling Kits, primary & secondary antibodies, streptavidin, phalloidins, and much more.

Frequently Asked Questions (FAQs) & Troubleshooting

Question	Answer
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.
Can I use a Mix-n-Stain™ kit for labeling proteins other than antibodies?	Mix-n-Stain™ kits are optimized for labeling IgG antibodies, but can be used to label other proteins. Customers have reported successful labeling of nanobodies and single chain antibodies. There are also published reports of Mix-n-Stain™ labeling of enzymes and lectins. However, Mix-n-Stain™ labeling conditions may cause denaturation of IgM antibodies. Note that any conjugation method, including Mix-n-Stain™, may affect the biological activity of proteins. Also, some free unreactive dye may remain after Mix-n-Stain™ labeling, which could interfere with live cell staining or trafficking studies using fluorescently labeled proteins. The ultrafiltration vial provided in the kit can be used to remove free dye after labeling if necessary. Additional ultrafiltration vials can be purchased separately (see Related Products).
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. See Considerations for Staining with Mix-n-Stain™ Labeled Antibodies.
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 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 (e.g. mouse-on-mouse).
What are the advantages of Mix-n-Stain™ kits over Zenon® antibody labeling kits from Thermo Fisher Scientific?	Zenon® conjugates use antibody fragments for labeling, while with Mix-n-Stain™ the dye is covalently attached to the antibody, which offers several advantages: 1. Covalent labeling eliminates the possibility of dye transfer or diffusion between antibodies during multi-color staining. 2. Unlike Zenon®, Mix-n-Stain labeling is not species-specific. 3. Mix-n-Stain™ conjugates are stable for at least 6 months in storage buffer, whereas Zenon® complexes must be used within 30 minutes. 4. Mix-n-Stain™ conjugates are less bulky because the dyes are directly linked to the antibody. 5. No post-staining fixation is required with Mix-n-Stain™.
What are the advantages of Mix-n-Stain™ kits over Expedeon 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 in Lightning Link® kits. Mix-n-Stain™ kits also are sized for labeling smaller amounts of antibody and are sold as single reactions, for greater flexibility.
What are CF® dyes?	CF® dyes are highly water soluble, small organic dyes for labeling proteins and nucleic acids. CF® dyes are designed to be brighter and more photostable than competing dyes. For more details, visit www.biotium.com.
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 ug, 2) 20-50 ug, and 3) 50-100 ug. For antibody labeling in the absence of stabilizer protein, select a kit that matches the amount of your antibody. For antibody labeling in the presence of stabilizer protein or ascites fluid, see Figure 1 or Table 1 of the product protocol.
If my antibody amount falls between two kits, which one should I use?	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.
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 in the labeling reaction?	The kits are optimized for labeling antibodies with a concentration between 0.5-1.0 mg/mL. Antibody concentrations outside the recommended range may result in either under- or over-labeling. See the protocol for instructions on how to concentrate or dilute the antibody if needed.
I performed immunofluorescence staining with my labeled antibody, but I don't see any signal. What should I do?	1. Check with the antibody manufacturer to confirm that the antibody formulation and concentration are compatible with the kit labeling protocol you selected. 2. 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. See Considerations for Staining with Mix-n-Stain™ Labeled Antibodies. 3. 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. 4. 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 ug) 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.