Product Information

Mix-n-Stain™ Antibody Labeling Kits

See product pages for product names, catalog numbers, and unit sizes for products included in this product information sheet:

Mix-n-Stain™ CF® Dye Antibody Labeling Kits

Mix-n-Stain™ Biotin Antibody Labeling Kit

Mix-n-Stain™ FITC Antibody Labeling Kit

Mix-n-Stain™ DNP Antibody Labeling Kit

Mix-n-Stain™ Digoxigenin Antibody Labeling Kit

Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits

Kit Contents

Component	5-20 ug labeling	20-50 ug labeling	50-100 ug labeling	3x(5-20 ug) labeling
Label vial*	1 vial Component A	1 vial Component A	1 vial Component A	3 vials Component A
Reaction Buffer, 10X	1 vial of 15 uL 99951-1	1 vial of 15 uL 99951-1	1 vial of 30 uL 99951	1 vial of 150 uL 99951-150UL
Antibody Storage Buffer	1 vial of 60 uL 99952-2	1 vial of 150 uL 99952-1	1 vial of 300 uL 99952	1 vial of 300 uL 99952
Ultrafiltration vial	1 each 99956	1 each 99956	1 each 99956	3 each 99956

***Note:** Mix-n-Stain™ labels are supplied as lyophilized solids. The amount of label in the vial is very small and usually is not visible. See FAQs on page 7 for details.

Storage and Handling

Store at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

Mix-n-Stain™ Antibody Labeling Kits contain everything you need to rapidly label an antibody with Biotium's next-generation CF® Dyes, other fluorophores, biotin, digoxigenin, or DNP. Simply mix your antibody with the Reaction Buffer and pre-measured label provided, followed by a brief incubation (Figure 1). Any free label is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. The antibody will be labeled with an average of 4-6 label molecules per antibody molecule.

Mix-n-Stain™ labeling is covalent, so labeled antibodies can be used for multiplex staining without transfer of the label between antibodies. We also offer Mix-n-Stain™ Antibody Labeling Kits for labeling IgM antibodies, nanobodies, or small ligands (see Related Products).

Kit Compatibility and Protocol Selection

The following are general considerations for kit compatibility and selecting the appropriate protocol for labeling. See Figure 2 and the Pre-Labeling Checklist for detailed kit compatibility and protocol selection guidelines. See pages 7-8 for troubleshooting tips and frequently asked questions (FAQs).

- Mix-n-Stain™ Antibody Labeling Kits are optimized for labeling IgG antibodies only. If you are labeling IgM antibodies, we recommend using our Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits (see Related Products).
- 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 (<10 kDa). Complete the Pre-Labeling Checklist on page 2 to find out whether you need to perform ultrafiltration before labeling.
- Labeling purified antibodies in PBS without stabilizer protein gives the best results. However, the standard Mix-n-Stain™ labeling protocol can be performed in the presence of up to 4-fold excess of BSA or gelatin to IgG (by ug amount). A modified protocol is provided for labeling antibodies with more than 4-fold excess BSA or gelatin, or antibodies in ascites fluid. Because CF® Dyes are highly water soluble, the presence of other proteins like BSA or gelatin in the labeling reaction has minimal effect on background fluorescence, because any stabilizer protein that is labeled will readily wash away during immunofluorescence staining.
- For an optimal degree of labeling (DOL, or label molecules per antibody molecule) and performance, we recommend an antibody amount in the middle of the kit range, for instance, 35 ug for the 20-50 ug kit. The Mix-n-Stain™ reaction, however, will tolerate an antibody amount at the lower or higher end of the range. If your antibody amount falls between two kit sizes, either kit should produce good results, but we recommend using the smaller kit size. We recommend the smaller kit because, in rare cases, antibody labeling can affect the binding affinity of monoclonal antibodies. A lower DOL may help avoid this. The modified protocol can also 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 optimal antibody concentration for labeling is 0.5-1 mg/mL. The ultrafiltration vial can be used to concentrate antibody solutions if needed. For quantitating antibodies of unknown concentration, Biotium offers the AccuOrange™ Protein Quantitation Kit, a highly sensitive fluorescence-based protein assay (see Related Products).

DOMINIQUE DUTSCHER SAS

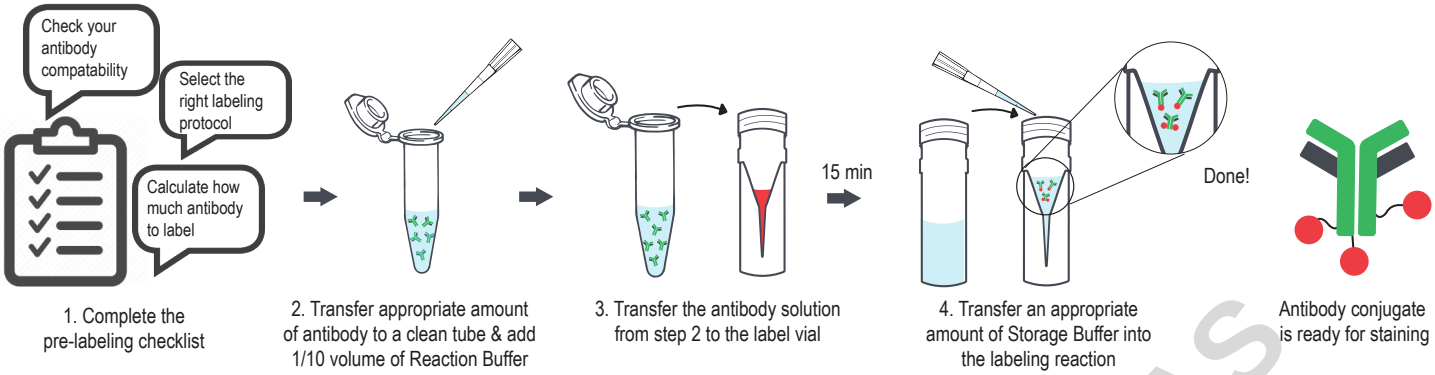


Figure 1. Mix-n-Stain™ antibody labeling overview.

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

The following are general considerations for using Mix-n-Stain™ Antibody Labeling Kits. See the Pre-Labeling Checklist on this page and step-by-step instructions starting on page 4 for use.

- Direct immunofluorescence with labeled primary antibodies may require a higher antibody concentration for similar staining intensity compared to indirect immunofluorescence detection with a secondary antibody. In our internal testing, indirect staining results in about 3-fold signal amplification compared to direct 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 in tissue sections and other samples. We also offer CF® Dye Tyramide Signal Amplification Kits, which can overcome background by amplifying immunofluorescence signal.
- Labeled secondary antibodies will still bind to primary antibodies labeled using Mix-n-Stain™ kits, therefore 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.

Before You Begin: Pre-Labeling Checklist

Mix-n-Stain™ Antibody Labeling Kits are very simple to use (see Figure 1). But before you begin, you must check that your antibody meets the compatibility requirements for labeling, and choose the right labeling protocol. We created this labeling checklist to walk you through each step of the way. We recommend printing the checklist to use as a worksheet each time you label a new antibody. See Figure 2 for an overview. We also provide checklists at the end of each labeling protocol that you can use as worksheets to keep track of each step during labeling.

1. Check the suitability of your antibody for direct labeling

1a) Have you tested this antibody in your application using a secondary antibody?

- ❑ Yes: Continue. Note that a higher antibody concentration may be required when using directly labeled antibodies compared to indirect detection.
- ❑ No: **Stop.** We recommend first testing your antibody with secondary antibody to verify that it shows robust and specific staining before attempting to directly label the antibody.

1b) Check the antibody isotype:

- ❑ IgG (any subclass): Continue
- ❑ IgM: **Stop.** This antibody isotype is not compatible with Mix-n-Stain™ labeling. IgM antibodies require a specialized labeling protocol. For labeling IgM antibodies, please see our Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits (Cat. No. 92558-92575). Contact Biotium's Technical Support team at techsupport@biotium.com to discuss further.
- ❑ IgA, IgD, or IgE: **Stop.** Read the related Frequently Asked Question (FAQ) on page 7 before continuing.

DOWNLOADED BY BIOTIUM SAS

2. Check the composition of your antibody

Important: If you don't know the antibody concentration or composition, contact the antibody supplier to get the answers to questions 2a-2e and 3b before trying to label your antibody.

2a) Does the antibody contain cell culture supernatant or crude serum?

- ❑ Yes: **Stop.** The antibody is not compatible with Mix-n-Stain™ labeling and requires purification before labeling using standard Protein A or Protein G purification protocols, or a commercially available antibody purification kit.
- ❑ No: Continue

2b) Does the antibody contain glycine, over 20 mM Tris, or over 10% glycerol?

- ❑ Yes: **Hold on.** You will need to perform ultrafiltration (Protocol A) before labeling to remove those substances. **But first, continue to question 2e.**
- ❑ No: Continue

2c) Is the antibody concentration below 0.5 mg/mL?

- ❑ Yes: **Hold on.** You will need to perform ultrafiltration before labeling to concentrate the antibody to 0.5-1 mg/mL (Protocol A). **But first, continue to question 2e.**
- ❑ No: Continue
- ❑ I don't know: **Hold on.** You will need to contact the antibody supplier to find out the antibody concentration before continuing.

2d) Is the antibody concentration above 1 mg/mL?

- ❑ Yes: **Hold on.** Dilute the antibody to 0.5-1 mg/mL with PBS.
- ❑ No: Continue

2e) Does the antibody contain BSA, gelatin, other proteins, or ascites fluid?

- ❑ Yes: Go to step 3
- ❑ No: Use Protocol B (Standard Labeling Protocol).
  Don't forget: If you answered yes to questions 2b or 2c, perform ultrafiltration (Protocol A) before labeling.

3. Check the ratio of antibody to other proteins

3a) Record the concentration of your antibody. If you do not know the concentration, contact the antibody supplier to find out the concentration before continuing.
Antibody (IgG) concentration (mg/mL): _____

3b) Record the concentration of other protein (e.g., BSA, gelatin, ascites, or protein). If you do not know the concentration, contact the antibody supplier to find out the concentration before continuing. You also can measure the OD_{280} of the solution to find the total protein concentration (IgG + other proteins).
Protein concentration (mg/mL): _____

3c) Calculate the ratio of protein to antibody
(protein concentration (3b) ÷ antibody concentration (3a))
Record the ratio here: _____

3d) Is the ratio of protein to antibody larger than 4?

- ❑ Yes: Use Protocol C (Modified Labeling Protocol)
- ❑ No: Use Protocol B (Standard Labeling Protocol)
  Don't forget: If you answered yes to questions 2b or 2c, perform ultrafiltration (Protocol A) before labeling.

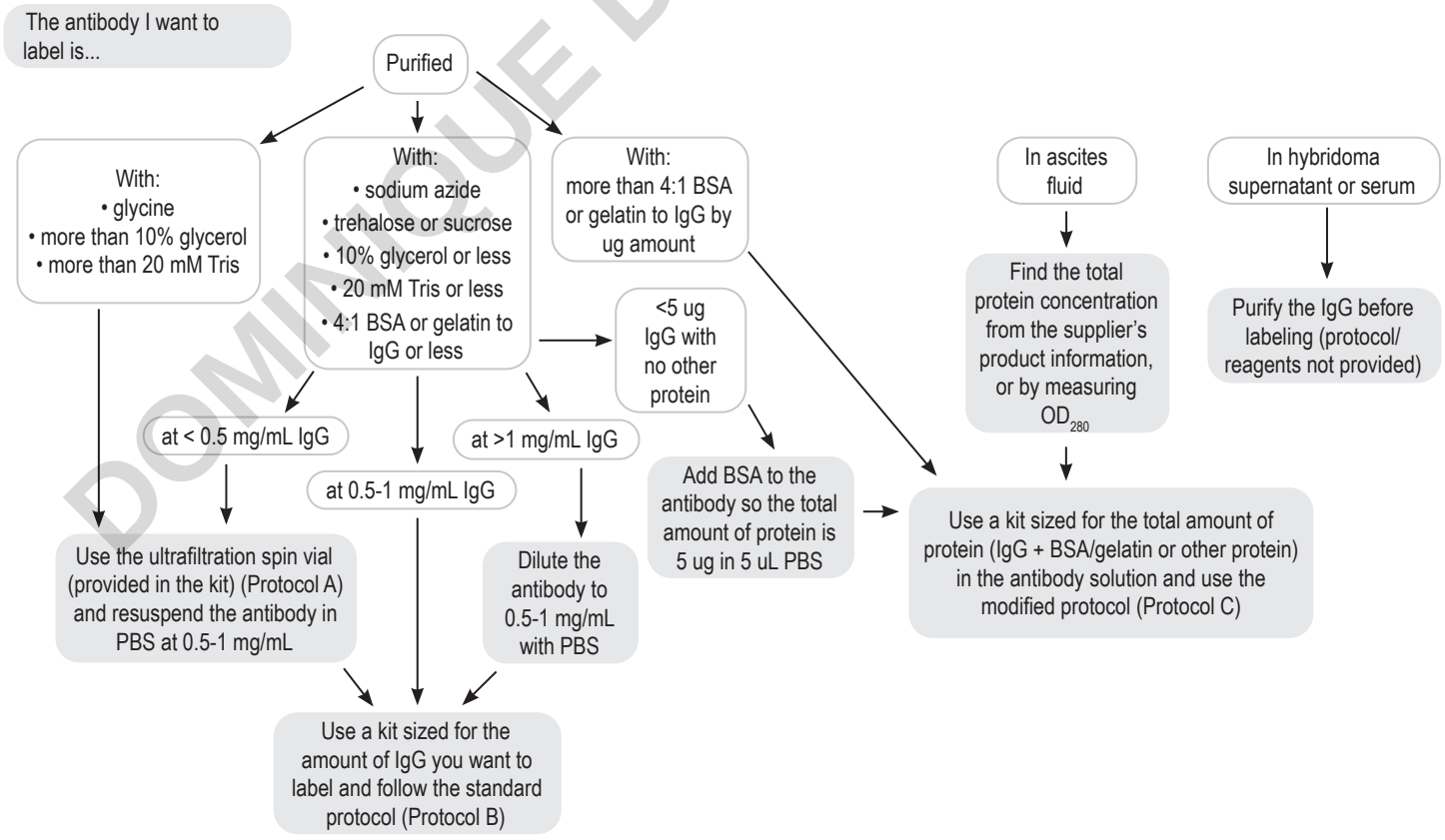


Figure 2. Mix-n-Stain™ compatibility and protocol selection overview.

DOMINIQUE DUTSCHER SAS

A. Ultrafiltration Protocol

Important: Before you begin, complete the Pre-Labeling Checklist (page 2) to determine whether your antibody requires ultrafiltration before labeling.

The ultrafiltration vial has a molecular weight cut-off of 10 kDa. 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 3). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

Note: Repeated filtration of large sample volumes (~500 uL) can lead to membrane failure. We therefore recommend keeping sample volumes at or below 350 uL.

Ultrafiltration Vial Capacities

- Maximum Sample Volume: 500 uL (see note above)
- 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. Centrifuge 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. Remove the flow-through liquid from the collection tube.

   Note: We recommend saving the filtrate solutions after Steps 1 and 2, so you can recover your antibody in case of membrane failure during centrifugation.
2. To concentrate your antibody, proceed to Step 3. To remove interfering substances, add an equal volume of 1X PBS to the membrane. Centrifuge the vial at 14,000 x g until the liquid has filtered into the collecting tube.
3. Add an appropriate volume 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 Protocol C (Modified Labeling Protocol), save the ultrafiltration vial to concentrate your antibody after labeling. Additional ultrafiltration vials also can be purchased separately (see Related Products).

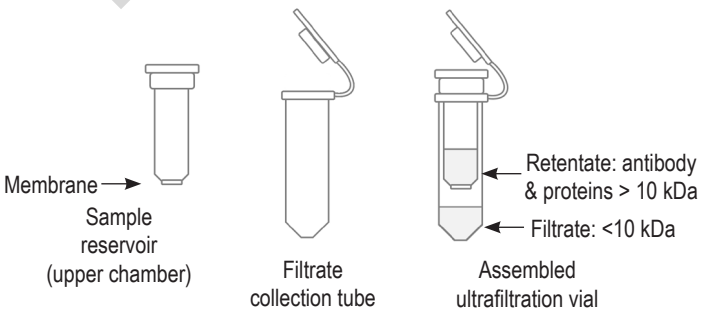


Figure 3. Ultrafiltration vial components.

B. Standard Mix-n-Stain™ Labeling Protocol

Important: Before you begin, complete the Pre-Labeling Checklist (page 2) to determine whether your antibody is compatible with labeling, and to choose the right labeling protocol. See page 5 for a worksheet that you can print and use to keep track of each step during labeling.

1. Start with your antibody at 0.5-1 mg/mL IgG in a compatible buffer. Transfer an appropriate amount of antibody to be labeled to a clean tube. See the Pre-Labeling Checklist for details.
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 every 9 uL of antibody solution, add 1 uL of 10X Reaction Buffer). Mix completely by pipetting up and down or gentle vortexing.

   Note: Adding Reaction Buffer is not optional. Labeling will not occur without it.
4. Transfer the entire solution from Step 3 to the vial containing the label (Component A). There is no need to measure the amount of the label in the vial. Vortex the vial for a few seconds and then briefly spin down contents in a centrifuge.
5. Incubate the vial in the dark for at least 15 minutes at room temperature. Incubating for longer times won't affect the labeling.
6. Dilute the labeled antibody solution with the provided Storage Buffer. Simply add the appropriate amount of Storage Buffer indicated in Table 1 below. 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.

Table 1. Storage Buffer Volume Required

Reaction Size	Storage Buffer
5-20 ug	60 uL
20-50 ug	150 uL
50-100 ug	300 uL

Note: Antibody Storage Buffer contains 2 mM sodium azide.

C. Modified Mix-n-Stain™ Labeling Protocol

Important: Before you begin, complete the Pre-Labeling Checklist (page 2) to determine whether your antibody is compatible with labeling, and to choose the right labeling protocol. See page 5 for a worksheet that you can print and use to keep track of each step during labeling.

1. Start with your antibody at 0.5-1 mg/mL total protein in a compatible buffer. Transfer an appropriate amount of antibody to be labeled to a clean tube. See the Pre-Labeling Checklist for details.
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.

DOMINIQUE DUTSCHER SAS

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 every 9 uL of antibody solution, add 1 uL of 10X Reaction Buffer). Mix completely by pipetting up and down or gentle vortexing.

   Note: Adding Reaction Buffer is not optional. Labeling will not occur without it.

4. Transfer the entire solution from Step 3 to the vial containing the label (Component A). There is no need to measure the amount of the label in the vial. Vortex the vial for a few seconds and then briefly spin down contents in a centrifuge.
5. Incubate the vial in the dark for at least 15 minutes at room temperature. Incubating for longer times won't affect the labeling.
6. Optional: You can add Storage Buffer to the reaction mixture as in Protocol B Step 6. However, this may result in a highly dilute IgG solution, which may not be practical for subsequent use. To concentrate the antibody before adding Storage Buffer, follow Steps 7-9 below.
7. Transfer the labeling reaction to the membrane of the ultrafiltration vial provided (or saved if Protocol A was performed for antibody clean-up). Centrifuge the vial at 14,000 x g until all of the liquid has filtered into the receiving vial as described in Protocol A.
8. Resuspend the labeled antibody in Storage Buffer or your preferred 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 storage.

Labeling Worksheets

Worksheet for Protocol B: Standard Labeling Protocol

1) Start with your antibody at 0.5-1 ug/uL (mg/mL) in compatible buffer (see Steps 1 & 2).
   Record the antibody concentration in ug/uL: _____

2) Check your kit size:
   - ❑ 5-20 ug
   - ❑ 20-50 ug
   - ❑ 50-100 ug

3) Choose a ug amount of antibody to label that falls within the kit range. For optimal DOL, we recommend an antibody amount in the middle of the kit range. Calculate the volume of antibody solution to use for labeling for the appropriate ug amount. Need help? See Calculations at the end of the worksheets.

❑ Transfer the volume of antibody solution calculated in Step 1 to a clean tube.
Record the input volume in uL: _____

❑ Add 1/10 volume of Reaction Buffer to the tube with antibody and mix well. For every 9 uL of antibody solution, add 1 uL of Reaction Buffer.

Note: Adding Reaction Buffer is not optional. Labeling will not occur without it.
Record the volume of Reaction Buffer added: _____

❑ Transfer the mixture of antibody and Reaction Buffer to the label vial and mix well. Incubate at least 15 minutes at room temperature, in the dark. After 15 minutes the reaction will have stopped, so incubating for longer times won't affect the labeling.

❑ Add the the appropriate volume of Storage Buffer to the reaction mixture. For 3x(5-20 ug) sizes, add 60 uL of Storage Buffer for each reaction.

❑ Calculate the final concentration of the labeled antibody (ug antibody input/uL total final volume). Need help? See Calculations at the end of the checklist.
Record final concentration: _____

☑ The labeled antibody is now ready to use.

Worksheet for Protocol C: Modified Labeling Protocol

1) Start with 0.5-1 ug/uL (mg/mL) total protein (IgG + other protein) in compatible buffer (see Steps 1 & 2). 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.
   Record the total protein concentration in ug/uL: _____

2) Check your kit size:
   - ❑ 5-20 ug
   - ❑ 20-50 ug
   - ❑ 50-100 ug

3) Choose a ug amount of total protein (IgG + other protein) to label that falls within your kit range. Calculate the volume of antibody solution to use for labeling for the appropriate ug amount determined by your kit size above. Need help? See Calculations at the end of the checklist.

❑ Transfer the appropriate volume of antibody or protein solution (from Step 1) to a clean tube.
Record the input volume in uL: _____

❑ Add 1/10 volume of Reaction Buffer to the tube with antibody and mix well. For every 9 uL of antibody solution, add 1 uL of Reaction Buffer.

Note: Adding Reaction Buffer is not optional. Labeling will not occur without it.
Record the volume of Reaction Buffer added: _____

❑ Transfer the mixture of protein and Reaction Buffer to the label vial and mix well. Incubate at least 15 minutes at room temperature, in the dark. After 15 minutes the reaction will have stopped, so incubating for longer times won't affect the labeling.

DOMINIQUE DUTSCHER SAS

❑ Optional: Use the ultrafiltration vial to concentrate the antibody. Resuspend in Storage Buffer or PBS with your preferred stabilizers or preservatives at a convenient concentration.

-or-

❑ Add the appropriate volume of Storage Buffer to the reaction mixture (see Table 1). For 3x(5-20 ug) sizes, add 60 uL of Storage Buffer for each reaction.

❑ Calculate the final concentration of the labeled antibody (ug antibody input/uL total final volume). Need help? See Calculations at the end of the checklist.
Record final antibody concentration: _____

☑ The labeled antibody is now ready to use.

Calculations

Protocol B: Volume of antibody solution to use for labeling:

$$\text{Volume to use (uL)} = \frac{\text{antibody amount (ug) that matches your kit}}{\text{antibody concentration (ug/uL)}}$$

Note: For optimal DOL, we recommend an antibody amount in the middle of the kit range.

Protocol C: Volume of antibody or protein solution to use for labeling:

$$\text{Volume to use (uL)} = \frac{\text{antibody amount (ug) that matches your kit}}{\text{total protein concentration (ug/uL)}}$$

Final concentration of antibody after labeling:

i. First calculate the final total volume:

Final total volume (uL) =
input volume + Reaction Buffer volume + Storage Buffer volume

ii. Then calculate the final concentration of antibody:

$$\text{Antibody concentration (ug/uL)} = \frac{\text{antibody input amount (ug)}}{\text{final total volume (uL)}}$$

Note: If using Protocol C, be sure to use the input amount of antibody for this calculation, not the input amount of total protein.

Troubleshooting Checklist

T1. No staining

T1a) Did you test this antibody with a secondary antibody before beginning to validate it?

❑ Yes: Continue

❑ No: **Stop.** We recommend first testing your antibody with secondary antibody to verify that it shows robust and specific staining before attempting to directly label the antibody.

T1b) Did you aliquot the label or try to label antibody input above or below the kit range?

❑ Yes: **Stop.** Mix-n-Stain™ kits are optimized for a single labeling reaction. We do not recommend trying to split the kit to label more than one antibody or for more than one use. Antibody input above or below the kit range will result in under- or over-labeling, which can result in poor staining or high background.

❑ No: Continue

T1c) Are your imaging or detection settings right for the label you're using?

❑ Check the excitation/emission maxima of your label in the technical data sheet which can be downloaded from the product page. You can also visit the CF® Dye technology page on our website to see which commonly used dyes are spectrally similar to your dye as an aid in choosing the right channel for detection.

Note: Far-red dyes (CF®633 and longer wavelengths) will not be visible through the microscope eyepieces, and must be imaged by confocal system or CCD camera. Blue dyes like CF®350, CF®405S, or CF®405M may be difficult to detect in tissue, which has high blue autofluorescence.

T1d) Are you using antifade mounting medium?

❑ Yes: Check the compatibility of your mounting medium. Some antifade media are not compatible with cyanine-based dyes like CF®647, CF®660C, CF®680, Cyanine 555, or Cyanine 647. Biotium's EverBrite™ Mounting Media (see Related Products) are compatible with a wide selection of dyes.

❑ No: We recommend using antifade fluorescence mounting medium with CF®350, which is less photostable than other dyes. Depending on the imaging method you are using, antifade may be needed for other dyes, particularly the cyanine-based dyes listed above. Most of our other CF® Dyes are highly photostable, visit the CF® Dye technology page on our website to learn more.

T1e) Can the labeled antibody be detected using a secondary antibody?

❑ Yes: Titrate the Mix-n-Stain™ labeled antibody; a higher antibody concentration may be required when using directly labeled antibodies compared to indirect detection, because the number of labels per target is lower.

❑ No: Continue

T1f) Did you perform ultrafiltration before labeling?

❑ Yes: Check to make sure the antibody was not lost to the flow-through due to a damaged membrane. The antibody can be recovered by performing ultrafiltration with a new ultrafiltration vial to concentrate the flow-through.

❑ No: Continue

T1g) Is your antibody monoclonal?

❑ Yes: Direct labeling can reduce antibody affinity for some monoclonal antibodies. If labeling adversely affects antibody binding, it may be necessary to use an alternative labeling chemistry for your antibody. Contact technical support through our website to discuss further.

❑ No: Contact technical support through our website to discuss further.

T2. Non-specific staining

T2a) Did you test this antibody with a secondary antibody before beginning to validate it?

❑ Yes: Continue

❑ No: **Stop.** We recommend first testing your antibody with secondary antibody to verify that it shows robust and specific staining before attempting to directly label the antibody.

DOMINIQUE DUTSCHER SAS

Troubleshooting Checklist (continued)

T2b) Did you aliquot the label or try to label antibody input above or below the kit range?

- ❑ Yes: **Stop.** Mix-n-Stain™ kits are optimized for a single labeling reaction. We do not recommend trying to split the kit to label more than one antibody or for more than one use. Antibody input above or below the kit range will result in under- or over-labeling, which can result in poor staining or high background.
- ❑ No: Continue

T2c) Is the non-specific binding seen only with Mix-n-Stain™ labeled antibody, but not unlabeled antibody + secondary antibody?

- ❑ Yes: Consider trying a blocking agent like TrueBlack® IF Background Suppressor (see Related Products) or Thermo's Image-iT® FX, which can block background arising from charge-based interaction of dyes with cells or tissues.
- ❑ No: Contact technical support through our website to discuss further.

Frequently Asked Questions (FAQs)

Question	Answer
How do I remove any unconjugated free label from the labeled antibody since there is no purification step?	Because of the unique formulations of our dyes and labeling technology, it is not necessary to remove unconjugated free label before staining. However, ultrafiltration can be used to remove free label if desired. We recommend performing ultrafiltration before adding antibody Storage Buffer.
Can I use antibodies labeled with Mix-n-Stain™ CF® Dye Antibody Labeling Kits for multi-color immunofluorescence staining, or will the dye transfer between antibodies?	Mix-n-Stain™ CF® Dye Antibody Labeling Kit labeling results in covalent linkage of dye and antibody, so there will be no dye diffusion or transfer between labeled antibodies or the target sample.
Can I use a Mix-n-Stain™ kit to label IgA, IgD, or IgE antibodies?	We haven't tested conjugating IgA, IgD, or IgE antibodies our Mix-n-Stain™ kits. Therefore, the kit user will need to optimize the labeling empirically. Our recommendation is to use the Mix-n-Stain™ CF® Dye Antibody Labeling Kit and start with a kit sized for half of the amount of IgA, IgD, or IgE antibody you are using. For example, use a 25-50 ug size kit with 100 ug IgA and adjust from there. This will achieve a lower degree of labeling, which is less likely to affect the antibody's function. If poor signal is observed, we recommend checking that the epitope binding was not affected by using a secondary antibody.
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 published reports of Mix-n-Stain™ labeling of enzymes and lectins. Biotium offers Mix-n-Stain™ kits designed specifically for optimal labeling of nanobodies (also called camelid or VHH domains), and small ligands. For other types of proteins, Biotium recommends the CF® Dye SE Protein Labeling kits (see Related Products). Any conjugation method, including Mix-n-Stain™, may affect the biological activity of proteins.
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 on page 2.
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 label is covalently attached to the antibody, which offers several advantages: 1. No possibility of label 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, whereas Zenon® complexes must be used within 30 minutes. 4. Mix-n-Stain™ conjugates are less bulky because the labels 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 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.

DOMINIQUE DUTSCHER SAS

Frequently Asked Questions (FAQs), continued

If my antibody amount falls between two kits, which one should I use?	Either kit size will produce good results, but we recommend using the smaller kit.
What label ratio should I use to ensure optimal labeling?	There is no need to measure the label amount or vary the reaction time as long as the amount of your antibody to be labeled falls within the kit's specified range.
How do I select a Mix-n-Stain™ kit?	For each CF® Dye, there are three types of 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. See the Pre-Labeling Checklist for details on how to select a kit to label antibody that contains BSA, gelatin, or ascites fluid. 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), then follow the Pre-Labeling Checklist.
Can I split the kit contents and use it more than one time?	No. The protocol we recommend is optimized for 1 labeling. We do not recommend that you try to split kit components to label more than one antibody or for more than one use. For multiple labeling reactions, see our Mix-n-Stain™ 3x(5-20) ug sizes.
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.
The Component A vial appears to be empty, should I ask for a replacement?	Mix-n-Stain™ labels are supplied as lyophilized solids. The amount of label in the vial is very small and usually is not visible. For green, red, and far-red dyes, the dye color will become visible when you mix your antibody solution into the vial. Blue dyes (CF®350, CF®405S, CF®405M) will appear colorless in solution. Non-fluorescent labels will not be visible.

Related Products

Cat. No.	Product
22004	Ultrafiltration vial, 10K MWCO (pack of 5)
22018	Ultrafiltration vial, 3K 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
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher
40083... 41037	NucSpot® Nuclear Stains
40061	RedDot™2 Far Red Nuclear Counterstain
23001-23002	EverBrite™ Mounting Medium (with or without DAPI)
23003-23004	EverBrite™ Hardset Mounting Medium (with or without DAPI)
23017-23018	EverBrite TrueBlack® Hardset Mounting Medium (with or without DAPI)
23008-23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23005	CoverGrip™ Coverslip Sealant
23023-23024	Super^{HT} PAP Pen 2.0
23006	Flow Cytometry Fixation/Permeabilization Kit
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer

Other Antibody or Protein Labeling Kits

Cat. No.	Product
92404... 92454	Mix-n-Stain™ Maxi Labeling Kits
92558... 92575	Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits
92500-92515	Mix-n-Stain™ Nanobody Labeling Kits
92549... 92557	Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits
92350-92364	Mix-n-Stain™ Small Ligand Labeling Kits
92294-92296	Mix-n-Stain™ FITC Antibody Labeling Kits
92412-92418	Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits
92244... 92444	Mix-n-Stain™ Biotin Antibody Labeling Kits
92328... 92450	Mix-n-Stain™ Digoxigenin Antibody Labeling Kits
92325-92327	Mix-n-Stain™ DNP Antibody Labeling Kits
92160-92163	VivoBrite™ Rapid Antibody Labeling Kits for Small Animal In Vivo Imaging
92210... 92228	CF® Dye & Biotin SE Protein Labeling Kits

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.

CF Dye, Mix-n-Stain, and modified Mix-n-Stain labeling are covered by US and international patents. Cubitainer is a registered trademark of Hedwin Corporation. Lightning-Link is a registered trademark of Abcam. Zenon and Image-iT are registered trademarks of Thermo Fisher Scientific.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

DOMINIQUE DUTSCHER SAS