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

See page 6 for kit catalog numbers and dye spectral properties.

***Note:** Mix-n-Stain™ dyes and 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 5 for details.

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 fluorophores, biotin, digoxigenin, or DNP. Simply mix your antibody with the reaction buffer and pre-measured dye provided, followed by a brief incubation (Figure 1). Any free dye or 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 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 (<10 kDa). Complete the pre-labeling checklist 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 four-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. The modified protocol 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. 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.

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).

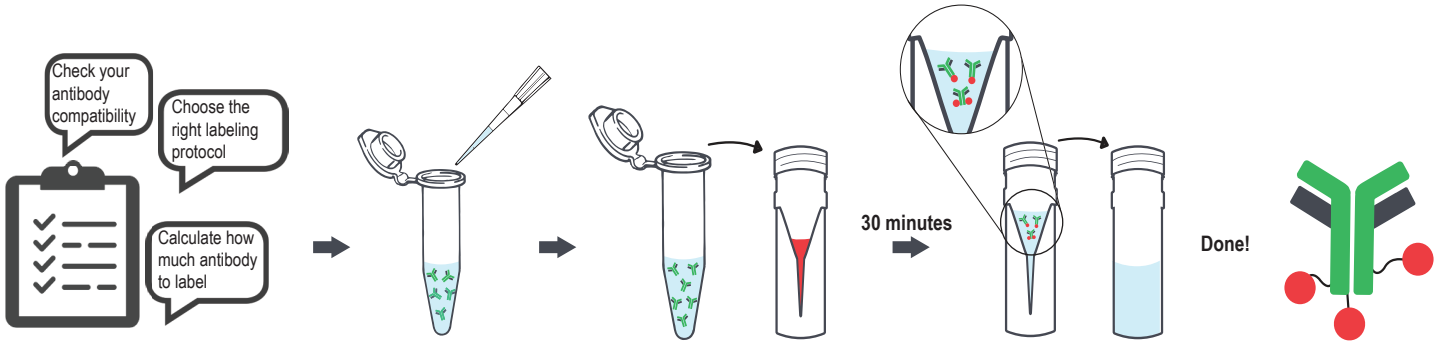
See Figure 2 and the Pre-Labeling Checklist for kit compatibility and protocol selection guidelines. See page 5 for frequently asked questions (FAQs) and troubleshooting tips.

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 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.



1. Complete the Pre-Labeling Checklist (page 2)

2. Pipette antibody to be labeled to a clean tube and add 1/10 volume of reaction buffer

3. Transfer the antibody solution from step 2 to the label vial and mix.

3. Mix the entire labeling reaction from step 3 into the vial of storage buffer.

Antibody conjugate is ready for staining.

Figure 1. Mix-n-Stain™ dye/hapten antibody labeling overview.

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 check list 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.

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.

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:

- ❑ Polyclonal antibody: Continue
- ❑ IgG (any subtype): Continue
- ❑ IgM: **Stop.** This antibody isotype is not compatible with Mix-n-Stain™ labeling. IgM antibodies require a specialized labeling protocol. Contact tech support through our website to discuss further.

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, more than 20 mM Tris, more than 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 (BSA/gelatin/ascites 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): _____

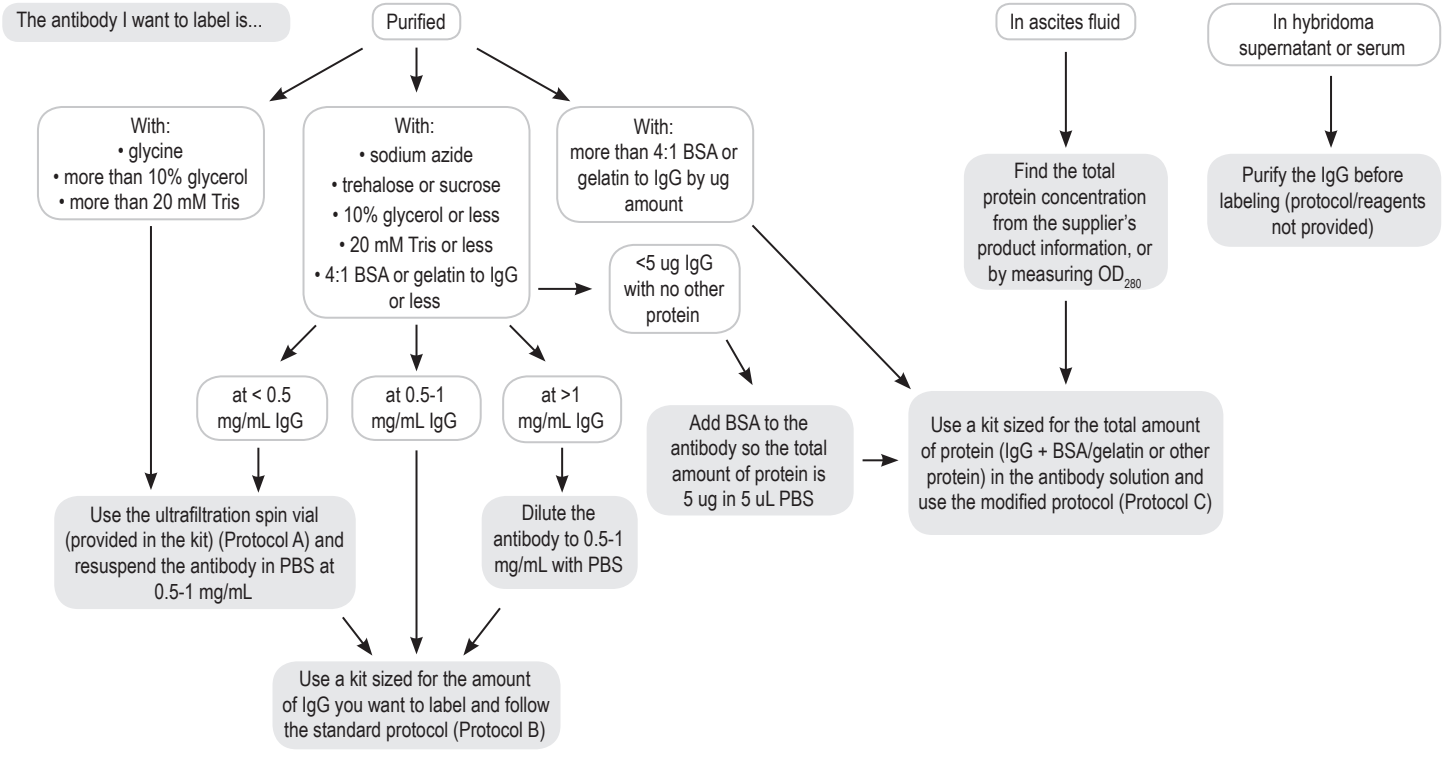
3c) Calculate the ratio of protein to antibody
(protein concentration (3b) ÷ antibody concentration (3a)
Record the ratio here: _____

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.



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 column has a molecular weight cut-off of 10,000. 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. Spin the vial at 14,000 x g until the liquid has filtered into the collecting 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 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).

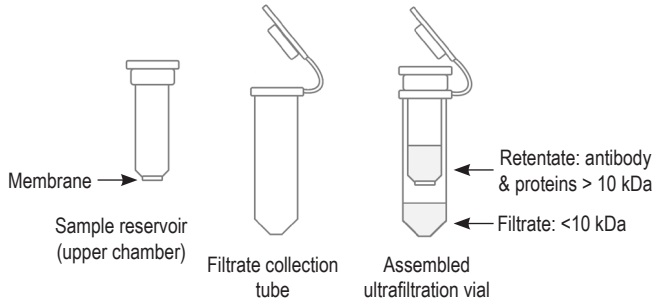


Figure 3. Ultrafiltration vial components.

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 4 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 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. Incubating for longer times won't affect the labeling.
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, 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 4 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.
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 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. Incubating for longer times won't affect the labeling.
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 steps 7-9 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.
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. 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 worksheets.

❑ Transfer the volume of antibody solution calculated in step 4c 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 dye vial and mix well. Incubate at least 30 minutes at room temperature, in the dark. After 30 minutes the reaction will have stopped, so incubating for longer times won't affect the labeling.

❑ Transfer the antibody/dye reaction mixture into the Storage Buffer vial and mix well.

❑ 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 your antibody at 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 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/protein solution (from step 5c) 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 dye vial and mix well. Incubate at least 30 minutes at room temperature, in the dark. After 30 minutes the reaction will have stopped, so incubating for longer times won't affect the labeling.

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

-or-

❑ Transfer the antibody/dye reaction mixture into the Storage Buffer vial and mix well.

❑ 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:

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

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

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

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 check question 1a in the Pre-Labeling Checklist on page 2?
- ❑ 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 dye 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 dye you're using?
- ❑ Check the excitation/emission maxima of your dye in Table 1 (page 6), or 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 dyes 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. You can check this as described in step d. 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.

Troubleshooting Checklist (continued)

T2. Non-specific staining

T2a) Did you check question 1a in the Pre-Labeling Checklist on page 2?

❑ 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.

T2b) Did you aliquot the dye 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 dye 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 dye before staining. However, ultrafiltration can be used to remove free dye if desired. We recommend performing ultrafiltration before adding antibody storage buffer.
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 between labeled antibodies or the target sample.
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. 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. An ultrafiltration vial with the correct molecular weight cut-off for your protein can be used to remove free dye after labeling if necessary (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. No 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, 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.
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. 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.
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 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.
The Component A vial appears to be empty, should I ask for a replacement?	Mix-n-Stain™ dyes and 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.

Table 1. Catalog Numbers and Spectral Properties

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®700	695/720	92425	92426	92427
CF®750	755/777	92284	92264	92241
CF®770	770/797	92285	92265	92242
CF®790	784/806	92288	92268	92248
CF®800	797/816	92428	92429	92430
CF®820	822/835	92431	92432	92433
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, 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
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
92404-92424	Mix-n-Stain™ Maxi 1 mg Scale Labeling Kits
92298-92299	Mix-n-Stain™ R-PE Antibody Labeling Kits
92306-92307	Mix-n-Stain™ APC Antibody Labeling Kits
92340-92341, 92346	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kits
92310-92311	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kits
92300-92302	Mix-n-Stain™ HRP Antibody Labeling Kits
92314-92315	Mix-n-Stain™ Alkaline Phosphatase Antibody Labeling Kits
92312-92313	Mix-n-Stain™ Glucose Oxidase Antibody 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.

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