

Product Information

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

Unit Size: One labeling reaction per kit

Kit Contents

Component	25 ug Labeling	100 ug Labeling
Dye vial*	1 vial Component A	1 vial Component A
Mix-n-Stain™ Reaction Buffer, 10X	15 uL 99951-1	30 uL 99951
Mix-n-Stain™ IgM Storage Buffer	200 uL 99875-200UL	780 mL 99875-780UL
Ultrafiltration vial (MWCO=10K)	1 each 99956	1 each 99956

*Mix-n-Stain™ dye is supplied as a lyophilized solid. The amount in the vial is very small and usually is not visible until solution is added. See FAQs (page 3) for additional information.

Storage and Handling

Store kit at -20°C. Kit components are stable for at least 12 months from date of receipt when stored as recommended.

Catalog Numbers & Spectral Properties

Dye	Ex/Em (nm)	Kit Size/Catalog Number	
		25 ug	100 ug
CF@405L	413/547	92558	92559
CF@405M	416/452	92560	92561
CF@488A	490/516	92562	92563
CF@555	554/568	92564	92565
CF@568	562/584	92566	92567
CF@594	593/615	92568	92569
CF@640R	642/663	92570	92571
CF@647	652/668	92572	92573
FITC	498/517	92574	92575

Product Description

IgM antibodies are difficult to label with fluorescent dyes because the conditions typically used for labeling IgG generally do not result in bright conjugates with good antibody reactivity for IgM. Biotium has resolved this issue by optimizing the reaction conditions for labeling IgM antibodies with our innovative Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits.

These kits are designed for labeling purified IgM antibodies with Biotium's bright and photostable CF® Dyes or FITC. The kits allow labeling of 25 ug or 100 ug of your IgM antibody in about 15-30 minutes, with minimal hands-on time and no purification after labeling.

Simply mix your purified IgM antibody with the reaction buffer and pre-measured dye provided, followed by a brief incubation. Any free dye or label in solution is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. The resulting conjugate is produced with optimal degree of labeling (DOL) for cell staining. Mix-n-Stain™ labeling is covalent, therefore labeled antibodies can be used for multiplex staining without the transfer of dye between proteins.

See page 3 for frequently asked questions (FAQs).

Considerations for Staining

Direct immunofluorescence staining should be done with high affinity antibodies against abundant targets. Antibodies should be validated using secondary detection before performing direct labeling.

Tissue staining by direct immunofluorescence can be challenging due to tissue autofluorescence and target integrity issues in human tissue. See our TrueBlack® line of background reducers (see Related Products) for reducing background in tissue sections and other samples. We also offer CF® Dye Tyramide Signal Amplification Kits, which can be used to amplify immunofluorescence signal to improve signal in tissue staining.

Considerations Before Use

The following are general considerations for kit compatibility.

- Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits are optimized for labeling IgM antibodies only. See our original Mix-n-Stain™ CF® Dye Antibody Labeling Kits for labeling IgG antibodies (see page 3).
- Unlike other Mix-n-Stain™ Antibody Labeling Kits, which can tolerate low levels of common buffer additives, Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits are designed specifically for purified IgM antibodies that are free of glycerol, gelatin, glycine, Tris, BSA, or other proteins. Using purified antibody solutions that are free of these additives is critical to achieving an optimal DOL. Check the compatibility of your antibody with the antibody compatibility guide on page 2 (Table 1). If your primary antibody is a commercial product, please contact the supplier to obtain the antibody concentration and formulation.
- These kits are optimized for a single labeling reaction of either 25 ug or 100 ug IgM antibody. We do not recommend splitting these kits for more than one labeling.
- If your antibody solution contains glycerol, Tris, or glycine, a microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove these incompatible small molecule buffer components (up to 10 kDa). Refer to the steps in Section A.
- If the antibody contains BSA or gelatin, or if the antibody is supplied as crude serum, ascites fluid, or hybridoma supernatant, purify the antibody prior to labeling using a protocol or a kit for IgM purification.
- If your antibody concentration is below 0.5 mg/mL, the ultrafiltration vial provided can be used to concentrate antibody solutions. For quantitating antibodies of unknown concentration, we offer the AccuOrange™ Protein Quantitation Kit, a highly sensitive fluorescence-based protein assay (see Related Products).

Mix-n-Stain™ Antibody Labeling Overview

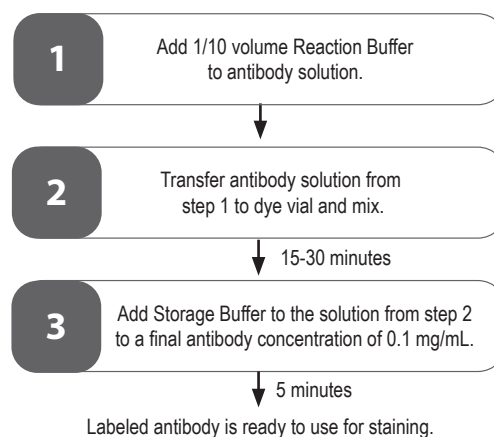


Table 1. Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits Compatibility and Labeling Protocol Selection Guide

Component	Compatibility
Sodium Azide	Compatible, proceed to Standard Labeling Protocol
Glycerol	Perform ultrafiltration before labeling (Section A)
Tris	Perform ultrafiltration before labeling (Section A)
Glycine	Perform ultrafiltration before labeling (Section A)
BSA	Not compatible, purify IgM
Gelatin	Not compatible, purify IgM
Ascites fluid	Not compatible, purify IgM
Serum	Not compatible, purify IgM
Hybridoma supernatant	Not compatible, purify IgM

A. Ultrafiltration Protocol

Important: Before you begin, use Table 1 to determine whether your IgM antibody requires ultrafiltration before labeling. If necessary, contact the manufacturer of your antibody to find out the concentration of antibody and stabilizers. If your antibody does not require ultrafiltration, proceed to the appropriate labeling protocol (see Table 1).

The ultrafiltration vial has a molecular weight cut-off of 10,000 Da. Molecules smaller than 10 kDa will flow through the membrane, and molecules larger than 10 kDa, including IgM antibodies, will be retained on the upper surface of the membrane (Figure 1). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody. Additional ultrafiltration vials also can be purchased separately (Cat. No. 22004).

Note: Repeated filtration of large sample volumes (~500 uL) can lead to membrane failure. We 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. Discard the liquid in the collection tube.
2. For IgM antibody concentration, proceed to Step 3. For clean-up, 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 filtrate receiving tube.
3. Add an appropriate concentration of PBS to the membrane to obtain a final protein concentration of 0.5 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. Proceed to the Standard Labeling Protocol.

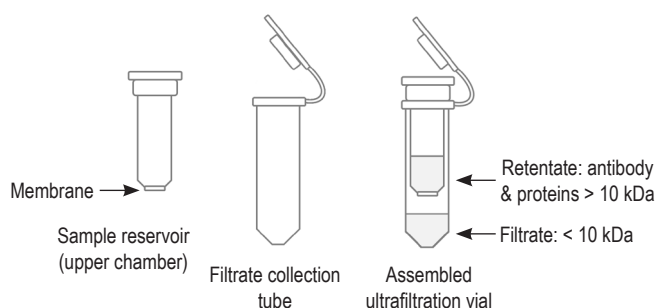


Figure 1. 10K MWCO ultrafiltration vial components.

B. Standard Labeling Protocol

1. Warm up the Mix-n-Stain™ Reaction Buffer and Storage Buffer vials to room temperature and vortex to mix before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Start with 25 ug and 100 ug IgM antibody (depending on the size of the kit) at 0.5 mg/mL in a compatible buffer. Transfer 50 uL (25 ug) or 200 uL (100 ug) of your antibody solution to be labeled to a clean tube.
3. Add the 10X Mix-n-Stain™ Reaction Buffer to the antibody solution at a ratio of 1:10, as shown below. Mix the solution completely by pipetting up and down or gentle vortexing:
 - a. For 25 ug labeling, add 5.5 uL of 10X Reaction Buffer to 50 uL of antibody.
 - b. For 100 ug labeling, add 22 uL of 10X Reaction Buffer to 200 uL antibody.

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 lyophilized dye (Component A). There is no need to measure the amount of the dye in the vial. Gently vortex for a few seconds to mix the solution.
5. Incubate the vial in the dark for 15-30 minutes. For best results we recommend incubating the reaction for the full 30 minutes.
6. Add Mix-n-Stain™ IgM Storage Buffer to the antibody solution. The resulting antibody concentration will be 0.1 mg/mL.
 - a. For 25 ug reaction, add 195 uL of Mix-n-Stain™ IgM Storage Buffer (Cat. No. 99875-200UL) to the solution in the dye vial and mix well.
 - b. For 100 ug reaction, transfer the solution from the dye vial directly into the vial of Mix-n-Stain™ Storage Buffer (Cat. No. 99875-780UL) and mix well.

Note: The Storage Buffer is required to terminate the labeling reaction, and is not optional.
7. Incubate the solution for at least 5 minutes in the dark at room temperature. The antibody is now ready to use for staining. The final IgM conjugate concentration is 0.1 mg/mL.

Storage of Labeled Antibodies

Labeled antibodies are stable for at least 6 months when stored at 4°C, protected from light.

Frequently Asked Questions (FAQs)

Question	Answer
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 remove the unconjugated free dye after labeling, 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 after labeling if desired.
Can I use Mix-n-Stain™ labeled antibodies for multi-color staining, or will the dye transfer between proteins?	Mix-n-Stain™ labeling results in covalent linkage of dye and antibody. At the end of the labeling reaction, no reactive dye remains, so there will be no transfer of dye to other proteins.
Can I use the kit for labeling proteins other than IgM antibodies?	Mix-n-Stain™ CF® Dye IgM Labeling Kits are specifically designed for labeling IgM antibodies. The kits could be used to label other proteins, but the number of dye molecules per protein molecule may not be optimal. Any conjugation method, including Mix-n-Stain™, may affect the biological activity of proteins. We also offer our original Mix-n-Stain™ Antibody Labeling Kits for labeling IgG antibodies, as well as Mix-n-Stain™ Nanobody Labeling Kits, for labeling Nanobodies®, and Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits, for labeling amine-functionalized compounds such as SNAP-tag®, CLIP-tag™, and HaloTag® ligands, or other molecules.
What size kit should I use?	The kit is designed for labeling precisely 25 ug or 100 ug antibody. We recommend aliquoting an appropriate amount of antibody at 0.5 mg/mL before labeling.
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 matches the amount that is 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 one labeling. We do not recommend that you try to split the kit to label for more than one reaction.
How important is the antibody concentration in the labeling reaction?	The kits are optimized for labeling antibodies at 0.5 mg/mL. Using higher or lower concentrations 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.

Related Products

Cat. No.	Product
22018	Ultrafiltration vial, 3K MWCO (pack of 5)
22004	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
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
40083... 41038	NucSpot® Nuclear Stains
40061	RedDot™2 Far Red Nuclear Counterstain
23008	Drop-n-Stain EverBrite™ Mounting Medium
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
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% solution
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder

Other Mix-n-Stain™ Labeling Kits

Cat. No.	Product
92330... 92463	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92244... 92444	Mix-n-Stain™ Biotin Antibody Labeling Kits
92294... 92411	Mix-n-Stain™ FITC Antibody Labeling Kits
92328... 92450	Mix-n-Stain™ Digoxigenin Antibody Labeling Kits
92325, 92326, 92327	Mix-n-Stain™ DNP Antibody Labeling Kits
92412...92419	Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits
92404... 92454	Mix-n-Stain™ Maxi Antibody Labeling Kits, 1 mg Labeling
92350-92364	Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits
92500-92515	Mix-n-Stain™ Nanobody Labeling Kits
92552 - 92550	Mix-n-Stain™ STORM CF® Dye 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 and secondary antibodies, streptavidin, phalloidins, and much more.

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