Revised: October 20, 2021

Product Information

Mix-n-Stain™ Maxi Antibody Labeling Kits

Catalog No.	CF® Dye	Ex/Em
92420	CF®350	347/448 nm
92421	CF®405S	404/431 nm
92404	CF®405M	408/452 nm
92454	CF®405L	395/545 nm
92405	CF®488A	490/515 nm
92406	CF®555	555/565 nm
92407	CF®568	562/583 nm
92408	CF®594	593/614 nm
92409	CF®633	630/650 nm
92410	CF®647	650/665 nm
92422	CF®680	681/698 nm
92423	CF®750	755/777 nm
92424	CF®770	770/797 nm
92411	FITC	494/518 nm
92415	Cyanine 555	555/565 nm
92419	Cyanine 647	650/665 nm

Kit Contents

Component	Size
Dye (Component A)	1 vial (lyophilized solid)
Mix-n-Stain™ Reaction Buffer 99951-150uL	150 uL
Mix-n-Stain™ Quenching Buffer 99854-150uL	150 uL

Unit Size: Single labeling reaction of 1 mg antibody per kit

Storage and Handling

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

Product Description

Mix-n-Stain™ Maxi Antibody Labeling Kits contain everything you need to rapidly label 1 mg of IgG antibody with Biotium's next-generation CF® Dyes or other fluorescent dyes. Simply mix your antibody with the Reaction Buffer and Dye provided, followed by a 30 minute incubation. After termination of the labeling reaction with the provided Quenching Buffer, the conjugate is ready to use (see Protocol Overview, next page). Any remaining free dye is no longer reactive at the end of the labeling, so the conjugate can be used for staining without further purification. Mix-n-Stain™ labeling is covalent, so labeled antibodies can be used for multiplex staining without transfer of dyes between antibodies.

Mix-n-Stain™ Maxi reactions are optimized for conjugation of 1 mg of IgG. After the reaction, the antibody will be labeled with an average of 4-6 dye molecules per antibody molecule. The kits can be used to label antibody fragments or other proteins, however, the degree of labeling (DOL, or number of dye molecules per antibody molecule) may not be optimal. The kits are not recommended for labeling IgM antibodies. See FAQs (page 3) for information on labeling nanobodies or other proteins.

These kits are not designed to be split for multiple labelings. For smaller labeling reactions, see our Mix-n-Stain™ CF® Dye Antibody Labeling Kits for labeling 5-20 ug, 20-50 ug, or 50-100 ug of antibody. Biotium also offers 3X(5-20 ug) sized kits for multiple labelings of 5-20 ug antibody.

We also offer Mix-n-Stain™ Antibody Labeling Kits for conjugating antibodies to fluorescent proteins (R-PE, APC, PerCP, and tandem dyes) or enzymes (HRP, AP, and glucose oxidase). Our CF® Dye SE Protein Labeling Kits are designed for performing conjugation with post-labeling purification. Visit www.biotium.com for more information.

See Frequently Asked Questions (FAQs) on page 3 for more information about Mix-n-Stain™ labeling technology.

Kit Compatibility and Considerations for Labeling

Antibodies should be at 1 mg/mL in PBS or similar buffer with no stabilizer proteins, glycerol, Tris, or amino acids such as glycine (See Table 1). Sodium azide does not affect the labeling.

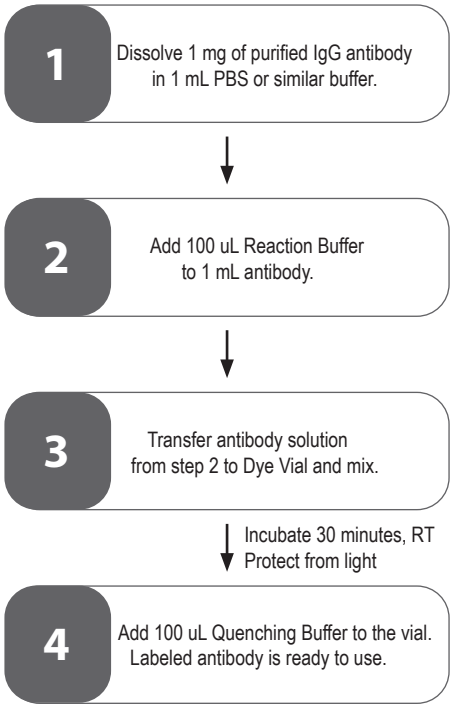
If your antibody concentration is higher than 1 mg/mL, add PBS to dilute it to 1 mg/mL. If your antibody concentration is lower than 1 mg/mL, concentrate the antibody to 1 mg/mL; a 10K MWCO ultrafiltration vial (see Related Products) can be used to concentrate your antibody. Ultrafiltration also can be used to remove incompatible small molecules such as glycerol, Tris, or glycine.

Antibodies containing protein stabilizers such as BSA or gelatin, or crude antibodies in serum, culture supernatant, or ascites fluid should be purified before labeling using a standard Protein A/G or similar purification protocol (ultrafiltration will not remove protein stabilizers).

Table 1. Reaction Compatibility Guide

Component	Compatibility
Sodium Azide	Compatible
Glycerol	≤ 10%: Compatible > 10%: Perform ultrafiltration (see Related Products)
Tris	≤ 20 mM: Compatible > 20 mM: Perform ultrafiltration (see Related Products)
Glycine	Perform ultrafiltration (see Related Products)
BSA or gelatin	Not compatible; purify antibody

Mix-n-Stain™ Maxi Protocol Overview



Related Products

Catalog number	Product
92500-92515	Mix-n-Stain™ Nanobody Labeling Kits
92437	Mix-n-Stain™ Maxi HRP Antibody Labeling Kit
22004	Ultrafiltration vial, 10K MWCO (pack of 5)
22018	Ultrafiltration vial, 3K MWCO (pack of 5)
22011	Fish Gelatin Powder
22013	Bovine Serum Albumin, Fraction V
22014	BSA, 30% Solution
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
40043	DAPI in H2O, 10 mg/mL
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
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X

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, phalloidin, and much more.

Labeling Protocol

Important**:** Before you begin, see Kit Compatibility and Considerations for Labeling and Table 1 to verify that your antibody is compatible with Mix-n-Stain™ Maxi labeling.

1. Prepare antibody for labeling at 1 mg/mL. If the antibody is in a lyophilized form or is more concentrated, reconstitute or dilute the antibody in PBS. Transfer 1 mL (1 mg) of antibody to a clean tube.
2. Warm up the Mix-n-Stain™ Reaction Buffer and the Mix-n-Stain™ Quenching Buffer to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
3. Add 100 uL of Mix-n-Stain™ Reaction Buffer to 1 mL antibody and mix well.
4. Transfer the entire antibody solution from Step 3 to the vial containing the dye (component A). There is no need to measure the amount of the dye in the vial. Vortex the vial for a few seconds to dissolve the dye.
5. Incubate the vial in the dark for 30 minutes at room temperature with gentle rocking.
6. To terminate the labeling reaction, add 100 uL of Quenching Buffer to the vial containing antibody and dye and mix well. The labeled antibody is now ready to use.

Storage of Labeled Antibody

The labeled antibody can be stored at 4°C, protected from light, for up to 3 days. For longer-term storage of labeled antibody, we recommend adding stabilizers to the antibody solution, such as sodium azide, BSA (see Related Products), and/or glycerol. Commonly used final concentrations of antibody stabilizers are 0.05% sodium azide, 2 mg/mL BSA or gelatin, and 50% glycerol. Antibodies with BSA and azide can be stored at 4°C, protected from light. Antibodies with BSA and 50% glycerol can be stored at -20°C, protected from light; the glycerol will prevent the solution from freezing, so it is not necessary to aliquot the antibody for storage.

Frequently Asked Questions (FAQs) & Troubleshooting

Question	Answer
Can Mix-n-Stain™ Antibody Labeling Kits be used to label nanobodies?	We offer Mix-n-Stain™ Nanobody Labeling Kits, specifically designed for optimized labeling of single-domain nanobodies with CF® Dyes. We also have had customers report successful labeling of nanobodies with our original CF® Dye Mix-n-Stain™ Antibody Labeling Kits. Mix-n-Stain™ kits are optimized for labeling whole IgG (150 kDa) with 4-6 dyes per antibody. Due to their smaller size (~15 kDa), single chain antibodies have fewer potential labeling sites. So to increase the degree of labeling for single chain antibodies, we recommend using a higher ratio of dye to protein. For example, the Mix-n-Stain™ 50-100 ug size kit could be used to label 25-30 ug of nanobody. Also, we do not recommend using the 10K MWCO ultrafiltration vial with proteins smaller than 30 kDa. If ultrafiltration is required prior to Mix-n-Stain™ labeling, a 3K MWCO ultrafiltration vial (Cat. no. 22018) should be used instead.
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.
How do I determine the degree of labeling (DOL) of my antibody after the reaction?	Mix-n-Stain™ kits are formulated to yield DOL of 4-6 dye molecules per antibody molecule. For ease-of-use and convenience, purification is not necessary before using the labeled antibody for staining. If you wish to measure DOL, you can use ultrafiltration (see Related Products) to purify the conjugate, then resuspend in PBS with no stabilizer proteins. For performing reactions to optimize DOL for your antibody or application, we recommend using our CF® Dye SE Protein Labeling Kits. The kits include everything you need to perform controlled conjugation reactions and post-labeling purification, as well as detailed instructions and correction factors for determining DOL for CF® Dyes.
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. Ultrafiltration vials can be used to remove free dye after labeling if necessary (see Related Products).
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 are sold as single reactions for multiple labeling scales between 5 ug and 1 mg, 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 different ranges: 5-20 ug, 20-50 ug, 50-100 ug, or 1 mg. Select a kit that matches the amount of your antibody. Mix-n-Stain CF® Dye kits for less than 1 mg antibody can be used for labeling antibody in the presence of excess stabilizer protein or ascites fluid using a modified protocol based on the total amount of protein present. See the relevant kit product information sheet for more information.
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 the kit.
Can I split the kit contents and use it more than one time?	No. The Mix-n-Stain™ kits are optimized for a single labeling reaction. 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?	Using a higher or lower antibody concentration may result in either under- or over-labeling. We recommend using antibody at 1 mg/mL for Mix-n-Stain™ Maxi labeling.