

# Product Information

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

**Unit Size:** Single labeling reaction of 50 ug antibody per kit

### Kit Contents:

Component	Size
Dye Vial (Component A)*	1 vial
99951-1: Mix-n-Stain™ Reaction Buffer, 10X	1 vial of 15 uL
99952-1: Mix-n-Stain™ Antibody Storage Buffer	1 vial of 150 uL
99956: Ultrafiltration vial (MWCO=10K)	1 each

\*STORM CF® Dyes are supplied as lyophilized solids. The amount of dye in the vial is very small and usually is not visible.

### Storage and Handling

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

### Catalog Numbers & Spectral Properties

Dye	Ex/Em (nm)	Cat. No.
CF@505	505/519	92549
CF@568	562/584	92550
CF@583R	585/609	92551
CF@597R	597/619	92553
CF@647	652/668	92554
CF@660C	667/685	92555
CF@680	681/698	92556
CF@750	755/779	92557

### Product Description

Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits contain everything you need to rapidly label an antibody with one of Biotium's STORM CF® Dyes. These next-generation fluorophores were developed with superior brightness and unique photoswitching properties that are ideal for super-resolution imaging by Stochastic Optical Reconstruction Microscopy (STORM). In addition, these specialized Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits are designed to result in a degree of labeling (DOL) of 1-2.5 molecules per antibody molecule, which is reported to be optimal for STORM (1). Simply mix your antibody with the reaction buffer and pre-measured dye provided, followed by a brief incubation. Any free dye is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. 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 at a higher DOL for applications such as flow cytometry, western blotting and conventional fluorescence microscopy (see Related Products).

### References

1) Proc. SPIE. 93310M (2015).

### Considerations Before Use

The following are general considerations for kit compatibility.

- Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits are optimized for labeling IgG antibodies. The labeling conditions may cause IgM antibodies to denature.
- Unlike other Mix-n-Stain™ Antibody Labeling Kits, which can tolerate low levels of common buffer additives, Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits are designed specifically for purified 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 the 1-2.5 DOL optimal for STORM imaging. Check the compatibility of your antibody with the antibody compatibility guide below (Table 1). If your primary antibody is a commercial product, please contact the supplier to obtain the antibody concentration and formulation.
- If your antibody solution contains glycerol, or less than 10 mM of Tris or amino acids, a microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove these incompatible small molecule buffer components (<10 kDa). Please refer to the steps in Section A.
- If the concentration of Tris or amino acids in your antibody solution is higher than 10 mM, we recommend using a buffer exchange column or dialysis to exchange the buffer for 1X PBS before labeling.
- If the antibody contains BSA or gelatin, or if the antibody is supplied as crude serum, ascites fluid, or hybridoma supernatant, purify the IgG prior to labeling using protein A purification or a commercial antibody clean-up kit, such as the Pierce Antibody Clean-Up Kit. Ultrafiltration will not remove stabilizer proteins or other cellular contaminants from antibody solutions.
- For an optimal DOL of 1-2.5, we strongly recommend using 50 ug of antibody with an accurate concentration of 1 mg/mL for labeling. Other antibody amounts and concentrations will result in variations in the DOL of the labeled conjugate.
- If your antibody concentration is below 1 mg/ml, the ultrafiltration vial provided can be used to concentrate antibody solutions. For quantitating antibodies of unknown concentration, Biotium offers the AccuOrange™ Protein Quantitation Kit, a highly sensitive fluorescence-based protein assay (see Related Products).

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

Component	Compatibility
Sodium Azide	Compatible, proceed to Standard Labeling Protocol
Glycerol	Perform ultrafiltration before labeling (Section A)
Less than 10 mM Tris or amino acids	Perform ultrafiltration before labeling (Section A)
Greater than 10 mM Tris or amino acids	Not compatible, perform buffer exchange or dialysis
BSA	Not compatible, purify IgG
Gelatin	Not compatible, purify IgG
Ascites fluid	Not compatible, purify IgG
Serum	Not compatible, purify IgG
Hybridoma Supernatant	Not compatible, purify IgG

## A. Ultrafiltration Protocol

**Important:** Before you begin please read the Considerations Before Use section and use Table 1 (Mix-n-Stain™ STORM CF® Dye Antibody Compatibility and Labeling Protocol Selection Guide) to determine whether your antibody requires ultrafiltration before labeling. If necessary, contact the manufacturer of your antibody to find out the concentration of IgG and antibody stabilizers. If your antibody does not require ultrafiltration or further purification, proceed to the labeling protocol (Section B).

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 IgG 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.

**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 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. Proceed to Section B

## B. Standard Mix-n-Stain™ Labeling Protocol

1. Start with at least 50 ug of antibody at 1 mg/mL IgG in a compatible buffer. Transfer 50 uL (50 ug) of your antibody solution to be labeled to a clean tube.

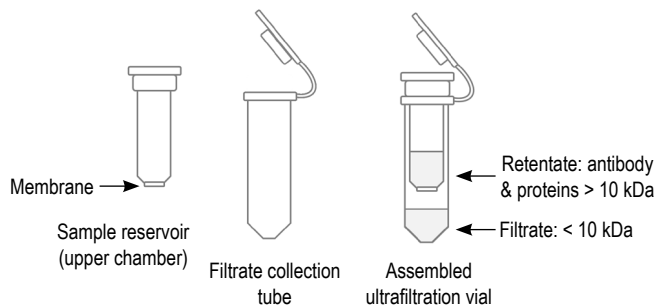


Figure 1. Ultrafiltration vial components.

2. Warm up the Mix-n-Stain™ Reaction Buffer vial and the Mix-n-Stain™ Storage Buffer vial to room temperature before use. Vortex and centrifuge the vials briefly to collect the solutions at the bottom of the vials.
3. Add 5 uL of the 10X Mix-n-Stain™ Reaction Buffer to the antibody solution so that the antibody solution contains a final concentration of 1X 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 (Component A). There is no need to measure the amount of the dye 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.

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

6. Transfer the entire volume of Storage Buffer to the reaction vial and vortex to mix. The antibody is now ready to use for staining. Alternatively, you can add the storage buffer of your choice.

**Note:** Antibody Storage Buffer contains 2 mM sodium azide.

## 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.

## Related Products

Cat. No.	Product
20792... 20825	Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
20793... 20826	Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
20794... 20827	Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
20795... 20828	Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
20829... 20836	Donkey Anti-Goat IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
20837- 20838	Donkey Anti-Guinea Pig IgG (H+L), Highly Cross-Adsorbed, CF® Dye Conjugates, Single Label for STORM
22004	Ultrafiltration vial, 10K MWCO (pack of 5)
22018	Ultrafiltration vial, 3K MWCO (pack of 5)
30092... 30104	MemBrite® Fix Cell Surface Staining Kits
40083... 41040	NucSpot® Nuclear Stains
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super <sup>HT</sup> Pap Pen 2.5 mm tip, ~400 uses
22006	Super <sup>HT</sup> Pap Pen 4 mm tip, ~800 uses
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

## Other Mix-n-Stain™ Antibody Labeling Kits

Cat. No.	Product
92230... 92463	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92404-92424, 92454	Mix-n-Stain™ Maxi Labeling Kits
92558-92575	Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits
92412... 92418	Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits
92294-92296	Mix-n-Stain™ FITC Antibody Labeling Kit
92244... 92444	Mix-n-Stain™ Biotin Antibody Labeling Kit
92325-92326	Mix-n-Stain™ DNP Antibody Labeling Kit
92328... 92450	Mix-n-Stain™ Digoxigenin Antibody Labeling Kits

Please visit [www.biotium.com](http://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.

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

CF Dye, Mix-n-Stain, and modified Mix-n-Stain labeling are covered by US and international patents.