SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Mix-n-Stain™ CF™594 Antibody Labeling Kits**

Catalog Numbers **MX594S20, MX594S50, and MX594S100**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

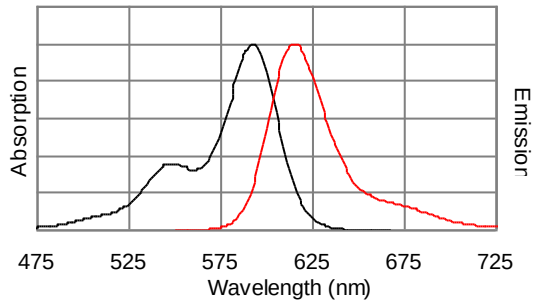
### Product Description

The kits contain everything needed to rapidly label an antibody. Select the labeling kit suitable for the amount of antibody to be labeled. The labeling procedure is simply mixing of the antibody with the optimally formulated dye in the reaction buffer provided, followed by a brief incubation. The resulting solution is ready for staining without further purification. The kit is suitable for labeling commercially available primary antibodies, either directly or after a simple antibody clean-up step. After labeling, the dye is covalently linked to the antibody with a degree of labeling of 4–6 dye molecules per antibody molecule. Multiple antibodies labeled with different dyes using Mix-n-Stain™ kits can be used for multicolor fluorescence staining without transfer of dyes between antibodies.

CF™594 is a deep red fluorescent dye spectrally similar to Alexa Fluor® 594 and Texas Red® dye. When conjugated to proteins, CF594 is significantly brighter than Alexa Fluor 594 due to its high quantum yield and exceptional water solubility. CF594 also has excellent photostability, making it ideal for demanding applications such as confocal microscopy and single molecule imaging. These properties make CF594 the best deep-red dye for labeling proteins and nucleic acids. The dye is particularly useful in combination with our blue fluorescent CF350, green fluorescent CF488A, and far red CF640R for multicolor imaging.

Note: Labeled secondary antibodies will bind to primary antibodies labeled using Mix-n-Stain kits; therefore, if multiple primary antibodies from the same species are to be used for multicolor immunofluorescence staining, a secondary antibody cannot be used to distinguish an unlabeled primary antibody from a Mix-n-Stain labeled primary antibody. Biotin Mix-n-Stain labeling kits are offered for secondary detection using CF dye-labeled streptavidin or CF dye-labeled monoclonal mouse anti-biotin.

**Figure 1.**
Absorption and emission spectra of CF594 conjugated to goat anti-mouse IgG in PBS.




CF594 dye properties:
Abs/Em Maxima: 593/614 nm
Flow cytometry laser line: 532 nm or 561 nm
Microscopy laser line: 594 nm
Direct replacement for: Alexa Fluor 594, DyLight® 594, and Texas Red

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store the kit at –20 °C.

### Procedures

A. Antibody Preparation
Check the compatibility of the antibody formulation with the compatibility table (see Table 1). An antibody solution free of stabilizers produces better labeling results. However, low levels of stabilizers can be tolerated in the standard protocol. Non-protein stabilizers can be removed using the ultrafiltration vial provided.

**Table 1.**
Mix-n-Stain Compatibility with Common Antibody Storage Components

| Component | Compatibility |
|---|---|
| Sodium Azide | compatible |
| Glycerol | ≤10%: proceed to Procedure C<br>>10%: proceed to Procedure B |
| Tris | ≤20 mM: proceed to Procedure C<br>>20 mM: proceed to Procedure B |
| Glycine | Proceed to Procedure B |
| BSA or gelatin | ≤4× IgG by weight: proceed to Procedure C<br>>4× IgG by weight: proceed to Procedure D |
| Ascites fluid | Proceed to Procedure D |
| Serum | Not compatible; purify IgG |
| Hybridoma supernatant | Not compatible; purify IgG |

Antibodies can be labeled in the presence of excess protein stabilizers or in ascites fluid by selecting a kit size that matches the amount of total protein in the labeling reaction (IgG plus stabilizer or amount of protein in ascites fluid) and using the modified Mix-n-Stain labeling procedure (Procedure D). The modified Mix-n-Stain protocol can be used to label antibody amounts that fall below the range of the kit by adding stabilizer protein to the antibody to bring the total protein amount within the kit range (see Procedure C).

One may experience higher background after staining if the antibody is labeled in the presence of BSA or gelatin. Background staining can be reduced by using blocking and wash solutions containing at least 1% BSA or gelatin, respectively. Alternatively, protein stabilizers (BSA or gelatin) can be removed using an antibody clean-up kit.

For optimal labeling, use the antibody at a concentration of 0.5–1 mg/mL. If necessary, adjust the antibody concentration with 1× PBS. The ultrafiltration protocol (Procedure B) can be performed to concentrate more dilute solutions of antibody.

B. Ultrafiltration

Notes: Before beginning, it is important to use Table 1 to determine whether the antibody requires ultrafiltration before labeling. If ultrafiltration is not required, proceed to the appropriate labeling procedure as determined by Table 1.

The ultrafiltration column membrane has a molecular mass cut-off of 10,000 Da. Therefore, 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. Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

Ultrafiltration vial capacities:
Maximum sample volume: 500 µL
Final concentrate volume: 15 µL
Filtrate receiver volume: 500 µL
Hold-up volume (membrane/support): <5 µL

1. Add an appropriate amount of antibody to the ultrafiltration vial being careful not to touch the membrane. Spin the solution at 14,000 × *g* in a microcentrifuge for a few minutes until the liquid is removed. Discard the liquid in the collection vessel.
2. Rinse the original antibody vial with an equal volume of 1× PBS and add to the ultrafiltration vial. Spin the vial at 14,000 × *g* until the liquid is removed. Resuspend the antibody to a concentration of 0.5–1 mg/mL in an appropriate volume of 1× PBS and transfer to a clean vial.
3. If using the modified Mix-n-Stain labeling procedure (Procedure D), save the ultrafiltration vial to concentrate the antibody after labeling.

C. Standard Mix-n-Stain Labeling
Note: Before beginning, use Table 1 to select the appropriate labeling protocol for the antibody.

1. 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.
2. Dilute the 10× Mix-n-Stain Reaction Buffer to 1× with the antibody solution. Mix by pipetting up and down a few times.
3. Transfer the entire solution from step 2 to the vial containing the CF dye (no need to weigh the dye). Vortex the vial for a few seconds.
4. Incubate the vial in the dark for 30 minutes. The solution is now ready to use. The concentration of the CF dye-labeled antibody conjugate is approximately the amount of the starting antibody divided by the total volume (i.e., ~100% labeling yield).
5. Dilute the labeled antibody solution with the provided Storage Buffer. Simply transfer the entire labeled antibody solution into the Storage Buffer and store at 2–8 °C. Recalculate the concentration of the antibody solution.
   Notes: Storage buffer contains 2 mM sodium azide.

   Alternatively, the labeled antibody solution can be aliquoted and stored at –20 °C. Without repeated freeze-thaws, the labeled antibody solution remains active for at least 6 months.

D. Modified Mix-n-Stain Labeling
Note: Before beginning, use Table 1 to select the appropriate labeling protocol for the antibody.

1. Use the antibody solution at a concentration of 0.5–1 mg/mL total protein (IgG plus stabilizer protein) for optimal labeling, using 1× PBS to dilute the solution if necessary. Make sure the amount of total protein (μg) in the labeling reaction falls within the range of the kit. 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 concentration.
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. Dilute the 10× Mix-n-Stain Reaction Buffer to 1× with the antibody solution. Mix by pipetting up and down a few times.
4. Transfer the entire solution from step 3 to the vial containing the CF dye (no need to weigh the dye). Vortex the vial for a few seconds.
5. Incubate the vial in the dark for 30 minutes.
   Optional: One 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 transfer the antibody to storage buffer without additional dilution, follow steps 6–8.
   Note: Storage buffer contains 2 mM sodium azide.
6. Transfer the labeling reaction to the membrane of the ultrafiltration vial provided (or saved from Procedure B). Centrifuge the vial at 14,000 × *g* until all of the liquid has filtered into the receiving vial as described in Procedure B.
7. Resuspend the labeled antibody in antibody storage 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: Storage buffer contains 2 mM sodium azide.
8. Transfer the recovered antibody solution to a fresh microcentrifuge tube. The antibody is now ready to use for staining. The labeled antibody is stable for at least 6 months when stored at 2–8 °C, protected from light. Alternatively, the antibody can be stored in single use aliquots at –20 °C for longer term storage. Without repeated freeze-thaws, the labeled antibody solution remains active for at least 6 months.