

# Product Information

## Mix-n-Stain™ Small Ligand Labeling Kits

**Size:** 1 labeling per kit

**Storage:** -20°C

**Stability:** Stable for at least 6 months from date of receipt when stored as recommended.

### Components:

Component	Amount
Reactive CF dye (component A)	1 vial
Reaction Buffer (#99999-20uL)	1 vial of 20 µL
Quenching Buffer (#99800-20uL)	1 vial of 20 µL
DMSO, anhydrous (#99953)	1 vial of 150 µL

### Product Application

Mix-n-Stain™ CF™ dye small ligand labeling kits are designed for rapid labeling of low molecular weight (Mwt ~ 150 - 5,000) and relatively high affinity biological ligands (or substrates) without a final purification step. The ligands to be labeled must contain an aliphatic amine group that is biologically inert. The amine group will form a covalent linkage with the provided reactive CF dyes. For example, suitable ligands or substrates include SNAP-tag®, CLIP-tag™, HaloTag® and TMP-PEG3-amine tag (Biotium Cat# 91056) derivatives that have an aliphatic amine. Many other small ligands are also possible candidates if they meet the criteria described above.

Biotium's CF dyes are a series of exceptionally bright and photostable water-soluble fluorescent dyes. The labeling procedure comprises simple mixing of small molecule ligand with the reaction buffer and the optimally formulated dye provided, followed by a brief incubation and quenching. No reactive Mix-n-Stain dye is available at the end of labeling; therefore the conjugate is ready for cell staining and imaging without further purification.

### Before You Begin

Mix-n-Stain small ligand labeling kits are optimized for labeling small ligands with an amino group (eg. SNAP- and CLIP-amine from New England Biolabs).

Preferably, the small molecule ligands should be dissolved in DMF or DMSO with minimal amount of water or aqueous buffer. The recommended concentration of the small molecule ligand for labeling is 5 – 50 mM. Over-diluted starting material may result in poor labeling efficiency and/or high background noise.

The protocol is designed to label 0.1 µmol small molecule ligand with reactive CF dyes. It is crucial to accurately determine the amount of the small molecule ligand to be labeled. It is highly recommended to (1) start from commercially available small molecule ligand which have been accurately aliquoted; or (2) measure the molar concentration of the small molecule ligand using UV-visible absorption spectroscopy.

### Mix-n-Stain Labeling Protocol

1. Warm up the components in the Mix-n-Stain labeling kits. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Prepare 10 mM stock solution of small molecule ligand in DMSO (eg, dissolve 1 µmol of the amino ligand in 100 µL DMSO), then take 10 µL of this solution to get 0.1 µmol of small molecule ligand.
3. Add 2 µL reaction buffer to the small molecule ligand solution. Mix by pipetting up and down or vortexing. Spin down the solution (Note 1).

4. Transfer the entire solution from Step 3 to the vial of CF dye. Gently vortex for 1 min.
5. Incubate the vial in the dark at room temperature for 30 min.
6. Add 2 µL quenching buffer to the solution. Vortex briefly to mix.
7. Incubate the vial in the dark at room temperature for 5 min.
8. The reaction mixture is ready for cell staining. Please refer to the user manual from the ligand supplier for protocols of cell staining and live cell imaging (Note 2). The concentration of CF dye-ligand conjugate is 0.1 µmol divided by the total volume. For convenience, the conjugate can be diluted to 1 mM for storage by adding DMSO to a total volume of 100 µL.
9. The CF dye-ligand conjugate can be stored in DMSO for at least 6 months at -20°C, protected from light. It is recommended to store and use the conjugate in small volume aliquots to avoid repeated freeze-thaws. Do not add any water or aqueous buffer for long term storage.

### Notes

1. If the primary amine is available as salt with acid, increase the amount of reaction buffer in Step 3 to up to 10 µL.
2. After live cell imaging the sample can be fixed and preserved for long term storage in our EverBright mounting medium. Fixed sample can also be stained with fluorophore conjugated antibodies.

### Ordering Information

Label/Dye	Ex (nm)	Em (nm)	Catalog Number	Staining
CF™405M	408	452	92362	Surface
CF™488A	490	515	92350	Surface
CF™568	562	583	92351	Surface
CF™594	593	614	92352	Surface
CF™633	630	650	92353	Surface
CF™640R	642	662	92354	Surface
CF™647	650	665	92359	Surface
CF™660C	667	685	92360	Surface
CF™680	681	698	92361	Surface
CF™680R	680	701	92355	Surface
CF™408	408	450	92356	Intracellular
CF™500	500	510	92357	Intracellular
CF™540	540	570	92358	Intracellular
CF™650	650	670	92363	Intracellular
CF™555	555	585	92364	Intracellular

For more information on Mix-n-Stain™ labeling technology, please see the Frequently Asked Questions section on page 2.

## Mix-n-Stain Small Ligand Labeling Kits Frequently Asked Questions

Question	Answer
What are the advantages of using small ligand-based chemical tags compared to fluorescent proteins for live cell imaging?	The chemical tags allow the target protein to be labeled with modular organic fluorophores. Compared to fluorescent proteins, our CF dyes benefits from smaller size, higher brightness and photostability, and unique photophysical properties that enable super-resolution imaging.
What are CF dyes?	CF dyes are highly water soluble, small organic dyes designed by scientists at Biotium for labeling proteins and nucleic acids. With a series of more than 20 colors, many of our CF dyes are brighter and more photostable than competing dyes. Several recent reports have demonstrated the superior performance of CF dyes in super-resolution and single-molecule imaging. Please see the product flyers for individual CF dyes and the CF dye brochure and FAQ at <a href="http://www.biotium.com">www.biotium.com</a> .
How to choose the right CF dyes for my experiment?	We provide a big variety of CF dyes for the Mix-n-Stain small ligand labeling kits. These kits are designed to label proteins either on cell surface or inside of live cells. To find the right kit for your experiment, first decide where the target protein would localize and then choose the desired color based on excitation and emission wavelengths.
How do I remove any unconjugated free dye from the labeled ligand since there is no purification step?	The unique formulations of our dyes and buffers and the labeling strategy have completely removed this concern. By the end of the labeling process, any unconjugated free dye would be inactivated so that it would not cause background staining. The exact mechanism on how this problem is solved is proprietary information.
Can I use Mix-n-Stain labeled small molecule ligand for multi-color imaging?	Mix-n-Stain labeling results in covalent linkage of dye and the ligand, so there will be no dye diffusion or transfer. Therefore it is ideal for multi-color imaging experiments.
Can I use a Mix-n-Stain kit for labeling molecules other than amino compounds?	Mix-n-Stain kits are optimized for labeling primary amines. We do not recommend them for labeling other functional groups.
I performed live cell imaging with my labeled ligand, but I don't see any signal. What should I do?	Check with the ligand manufacturer to confirm that the ligand structure and concentration are compatible with the kit labeling protocol you selected. The covalent conjugation of the CF dyes to the ligand can be monitored by running thin layer chromatography (TLC). For live cell imaging using chemical tags, you should confirm your target protein-receptor fusion protein is properly constructed and expressed. Co-transfection with indicating vector (eg. vector expressing fluorescent proteins) and western blot may help to determine the efficiency of transfection and protein expression.

<sup>1</sup> F. Görlitz et al, S.W. Hell, *Prog Electromagn Res*, **2014**, 147, 57.

<sup>2</sup> P.J. Bosch et al, V. Subramaniam, *Biophys J*, **2014**, 107, 803.

## Related products

Catalog #	Product Name	Unit Size	Catalog #	Product Name	Unit Size
23001	EverBrite Mounting Medium	10 mL	40044	Hoechst 33258, 10 mg/mL in H <sub>2</sub> O	10 mL
23002	EverBrite Mounting Medium with DAPI	10 mL	40046	Hoechst 33342, 10 mg/mL in H <sub>2</sub> O	10 mL
23003	EverBrite Hardset Mounting Medium	10 mL	40043	DAPI, 10 mg/mL in H <sub>2</sub> O	1 mL
23004	EverBrite Hardset Mounting Medium with DAPI	10 mL	40060	RedDot 1, 200X	250 µL
21003	One-Step Blue Protein Gel Stain	1L	70054	MitoView Green	20 X 50 µg
21004	One-Step Lumitein Protein Gel Stain	1L	70055	MitoView 633	20 X 50 µg
21005	One-Step Lumitein UV Protein Gel Stain	1L	60010	Dil (or DiIC18(3))	50 mg
			91056	TMP-PEG3-amine, TFA salt	1 mg

Please visit [www.biotium.com](http://www.biotium.com) to view our full selection of products featuring bright and photostable CF™ dyes, including secondary antibodies, streptavidin, anti-biotin, and anti-tag antibodies. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes, fluorescent Annexin V conjugates, and NucView™488 Caspase-3 Substrate for live cells.

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