

Product Information

Mix-n-Stain™ CF™640R antibody labeling kit, 1 labeling

Catalog Number: 92245, 50 - 100 ug labeling

Components and Storage:

Material	Quantity	Storage	Stability
CF640R dye (#92245A)	1 vial	-20 °C	Stable for 3 months from time of reception if stored as directed.
Mix-n-Stain reaction buffer, 10X (#99951)	1 vial of 30 uL		
Mix-n-Stain storage buffer (#99952)	1 vial of 300 uL		
Ultrafiltration vial (MWCO=10K) (#99956-1)	1 vial		

Catalog Number: 92258, 20 - 50 ug labeling

Components and Storage:

Material	Quantity	Storage	Stability
CF640R dye (#92258A)	1 vial	-20 °C	Stable for 3 months from time of reception if stored as directed.
Mix-n-Stain reaction buffer, 10X (#99951-1)	1 vial of 15 uL		
Mix-n-Stain storage buffer (#99952-1)	1 vial of 150 uL		
Ultrafiltration vial (MWCO=10K) (#99956-1)	1 vial		

Catalog Number: 92278, 5 - 20 ug labeling

Components and Storage:

Material	Quantity	Storage	Stability
CF640R dye (#92278A)	1 vial	-20 °C	Stable for 3 months from time of reception if stored as directed.
Mix-n-Stain reaction buffer, 10X (#99951-1)	1 vial of 15 uL		
Mix-n-Stain storage buffer (#99952-2)	1 vial of 60 uL		
Ultrafiltration vial (MWCO=10K) (#99956-1)	1 vial		

Technical Summary

Abs/Em Maxima: 642/662 nm (See Figure 1)

Flow cytometry laser line: 633, 635 or 640 nm

Microscopy laser line: 633, 635, 640 or 647 nm

Direct replacement for: Alexa Fluor® 647, Cy™5, DyLight™649

Product Application

This kit contains everything you need to rapidly label an antibody. Please select the labeling kit suitable for the amount of antibody you wish to label. The labeling procedure comprises simple mixing of your antibody and 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.

CF™640R is a novel rhodamine-based far-red dye with excellent fluorescence quantum yield and exceptional photostability. The dye can be efficiently excited by the 633, 635, 640 or 647 nm laser with emission maximum at 650 nm. Both the excitation and emission wavelengths of CF™640R are only slightly shorter than those of the cyanine-based dye Cy™5 or Alexa Fluor®647 (Figure 1). Thus, CF™640R can be directly used to replace Alexa Fluor® 647 or Cy™5 in any application. CF™640R is much brighter than Cy™5 and at least as bright as Alexa Fluor® 647 when detected using optical settings optimized for either of the latter two dyes. The major benefit of using CF™640R, however, is the dye's superior photostability, which is simply unmatched by any cyanine-based far-red dyes, such as Alexa Fluor®647 and Cy™5.

For more information on CF™640R and other far-red CF™ dyes, please visit the Biotium website.

Protocol

Before you begin:

a) Check the compatibility of your antibody with the antibody compatibility guide on the next page. Low levels of BSA, gelatin, Tris, glycerol or sodium azide in the antibody solution can be tolerated. However, if the amount of stabilizers are above the threshold concentrations, use either a protein A purification procedure or a commercial antibody clean-up kit, such as the Pierce antibody clean-up kit (cat#44600) to remove protein stabilizers. An antibody solution free of stabilizers produces better labeling results. If you need to remove just non-protein components such as Tris, glycine or glycerol, use the ultrafiltration vial provided in the kit to purify your antibody by following the steps below. If no clean-up is required, proceed to step b).

1. Add an appropriate amount of antibody to the ultrafiltration vial being careful not to touch the membrane. Spin the solution at max speed (14,000 rpm) 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 1X PBS and add to the ultrafiltration vial. Spin the vial at max speed until the liquid is removed. Resuspend the antibody in an appropriate amount of 1X PBS to derive a concentration of 0.5 - 1 mg/mL.

b) Use your antibody at a concentration of 0.5-1 mg/mL for optimal labeling. If the antibody is in a lyophilized form or is more concentrated, reconstitute or dilute the antibody in PBS. If your primary antibody is a commercial product with no quantity information, please contact the supplier. Resuspend the antibody in an appropriate amount of 1X PBS.

c) 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 concentrate the solutions to the bottom of the vials.

Procedure:

1. Dilute the 10X Mix-n-Stain™ Reaction Buffer to 1X in the antibody solution. Mix the solutions by pipetting up and down a few times.
2. Transfer the entire solution from Step 1 to the vial containing the CF™ dye (no need to measure the amount of the dye). Vortex the vial for a few seconds.
3. Incubate the vial in the dark for 30 minutes. The solution is now ready to use. The concentration of the CF dye-labeled antibody is approximately the amount of your starting antibody divided by the total volume (*i.e.*, ~100% labeling yield).
4. Dilute the labeled antibody solution with the provided Storage Buffer. Simply transfer the entire labeled antibody solution into the Storage Buffer and store at 4 °C. Recalculate the concentration of the antibody solution. Alternatively, you can aliquot the solution and store at -20 °C. Without repeated freeze-thaws, the shelf-life of the labeled antibody solution should be at least 6 months.

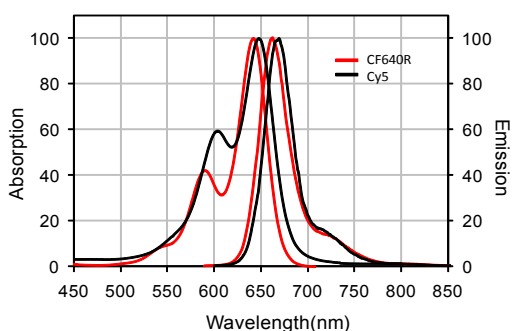


Figure 1. Absorption and emission spectra of CF™640R and Cy™5 conjugated to goat anti-mouse IgG in PBS.

Mix-n-Stain™ Compatibility with Common Antibody Storage Components

Component	Compatibility
BSA or gelatin	< 4 equivalent of antibody by weight
Glycerol	< 10%
Glycine	not compatible
Serum	not compatible
Sodium Azide	compatible
Tris	< 20 mM

* If your antibody contains storage buffer components above the compatibility threshold levels, purify your antibody.

** An antibody solution free of protein stabilizers produces the best results. Although low levels of BSA and gelatin can be tolerated, you may experience slightly higher background after staining if your antibody was labeled in the presence of these proteins.

*** If the antibody was labeled in the presence of BSA or gelatin, background staining can be greatly reduced by using blocking and wash solutions containing at least 1% BSA or gelatin, respectively.

Related Products

Cat.#	Product Name	Unit Size
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
22010	10X Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 X 50 g
22010	Dry Milk Powder	4 X 25 g

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

Dye	Packaging	Cat. No.	Packaging	Cat. No.	Packaging	Cat. No.
CF™350	1 X (50-100 ug) labeling	92230	1 X (20-50 ug) labeling	92250	1 X (5-20 ug) labeling	92270
CF™405S	1 X (50-100 ug) labeling	92231	1 X (20-50 ug) labeling	92251	1 X (5-20 ug) labeling	92271
CF™405M	1 X (50-100 ug) labeling	92232	1 X (20-50 ug) labeling	92252	1 X (5-20 ug) labeling	92272
CF™488A	1 X (50-100 ug) labeling	92233	1 X (20-50 ug) labeling	92253	1 X (5-20 ug) labeling	92273
CF™555	1 X (50-100 ug) labeling	92234	1 X (20-50 ug) labeling	92254	1 X (5-20 ug) labeling	92274
CF™568	1 X (50-100 ug) labeling	92235	1 X (20-50 ug) labeling	92255	1 X (5-20 ug) labeling	92275
CF™594	1 X (50-100 ug) labeling	92236	1 X (20-50 ug) labeling	92256	1 X (5-20 ug) labeling	92276
CF™633	1 X (50-100 ug) labeling	92237	1 X (20-50 ug) labeling	92257	1 X (5-20 ug) labeling	92277
CF™640R	1 X (50-100 ug) labeling	92245	1 X (20-50 ug) labeling	92258	1 X (5-20 ug) labeling	92278
CF™647	1 X (50-100 ug) labeling	92238	1 X (20-50 ug) labeling	92259	1 X (5-20 ug) labeling	92279
CF™660C	1 X (50-100 ug) labeling	92239	1 X (20-50 ug) labeling	92260	1 X (5-20 ug) labeling	92280
CF™660R	1 X (50-100 ug) labeling	92243	1 X (20-50 ug) labeling	92261	1 X (5-20 ug) labeling	92281
CF™680	1 X (50-100 ug) labeling	92240	1 X (20-50 ug) labeling	92262	1 X (5-20 ug) labeling	92282
CF™680R	1 X (50-100 ug) labeling	92246	1 X (20-50 ug) labeling	92263	1 X (5-20 ug) labeling	92283
CF™750	1 X (50-100 ug) labeling	92241	1 X (20-50 ug) labeling	92264	1 X (5-20 ug) labeling	92284
CF™770	1 X (50-100 ug) labeling	92242	1 X (20-50 ug) labeling	92265	1 X (5-20 ug) labeling	92285

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