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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

CF™640R Goat Anti-Mouse IgG, F(ab')2 Fragment, Fc gamma Fragment Specific, highly cross-adsorbed

Unit Size: 500 ug

Concentration
Lyophilized format (after reconstitution): 1 mg/mL in pH ~7.4 PBS containing 15 mg/mL bovine serum albumin (IgG-free and protease-free) and 20 mg/mL trehalose.

Color and Form
Blue solid

Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 642/662 nm (in pH 7.4 PBS buffer)

CF™640R is spectrally similar to AlexaFluor® 647, Cy® 5, and DyLight® 649.

Storage and Handling
Store at -20°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Reconstitution: add 0.5 mL dH_2O and mix gently to dissolve. Store at -20°C, protected from light. Aliquot to avoid freeze-thaw cycles. Alternatively, glycerol can be added to the antibody so that it will not freeze at -20°C: add 0.25 mL dH_2O to the lyophilized antibody and mix gently to dissolve, then add 0.25 mL glycerol and mix well. Optional: a preservative may be added, such as 0.05% (final concentration) sodium azide.

Note: storage of the antibody for more than a day at final working dilution is not recommended.

Product Description

This product is prepared by labeling high quality F(ab')2 fragment goat anti-mouse IgG, Fcγ fragment specific, with the far-red fluorescent dye CF™640R. The antibody has been adsorbed against human, bovine and horse serum to minimize cross-reactivity.

CF™640R is a rhodamine-based far-red fluorescent dye with excitation and emission maxima very similar to those of Cy®5 and AlexaFluor® 647 (Figure 1). CF™640R is much brighter than Cy®5 and at least as bright as AlexaFluor® 647. A major advantage of CF™640R is that it is exceptional photostable, unlike the cyanine based dyes Cy®5 and Alexa Fluor® 647 which, like other cyanine dyes in general, have intrinsically poor photostability. CF™640R is also brighter and more photostable than Atto 647N, another spectrally similar dye frequently used in single-molecule imaging. The combination of excellent brightness and photostability makes CF™640R ideal for confocal microscopy, single-molecule imaging and other demanding applications based on fluorescence detection. Please see the CF™640R flyer at www.biotium.com for more information.

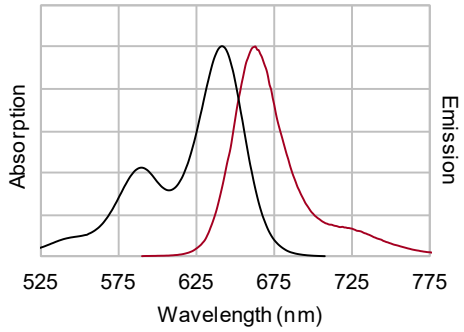

Figure 1. Absorption and emission spectra of CF™640R antibody conjugates.

General Protocols for Using CF™-Dye Labeled Secondary Antibodies

Recommended Dilution Range

1-10 μg/mL of the IgG conjugate for most applications (appropriate dilutions of the conjugate should be determined empirically). See other side for example staining protocols.

Immunofluorescence Protocol for Microscopy

There are many methods for immunofluorescence staining. The protocol below is a general guideline for staining cells and should be optimized or modified to obtain the best results for each particular application.

1. Coverslip preparation for adherent cells

1.1 Culture cells on slide chambers or sterile glass coverslips (with poly-L-lysine coating if cells do not adhere well, see below). We recommend 18 x 18 mm square coverslips in 6-well plates or 4-well chamber slides.
1.2 Allow cells to adhere and treat as desired.
1.3 Rinse cells gently with PBS.

2. Coverslip preparation for non-adherent cells

2.1 Coat coverslips with 0.01% poly-L-lysine solution for 10 minutes at room temperature.
2.2 Aspirate the poly-L-lysine solution and allow coverslips to dry completely.
2.3 Centrifuge cells in medium and resuspend in PBS. Transfer cells to coverslips.
2.4 Incubate for 30-60 minutes. Check for adherence by microscope.

3. Fixation and Staining

3.1 Fix with 4% paraformaldehyde/PBS, 15 min.
3.2 Rinse twice with PBS to remove traces of fixative.
3.3 Permeabilize with 0.1 - 0.5% TritonX-100/PBS, 5-10 min.
3.4 Block with blocking agent such as with 5% BSA or normal goat serum in PBS, 30 min.
3.5 Dilute primary antibody in dilution buffer as recommended in the specific product's datasheet. Overlay enough diluted antibody to cover cells on coverslip (150-200 μL is usually sufficient to cover the surface area) or add to each chamber of the chamber slides. Keep slips covered or in a humidified chamber to avoid evaporation.
3.6 Rinse three times with PBS, 5 min each wash.
3.7 Dilute fluorescent secondary antibody in dilution buffer and incubate for 1 hour at room temperature. General range for IgG conjugates is between 1-10 μg/mL for most applications. Cell samples without primary antibody incubation is recommended for background control. Keep slips covered or in a humidified chamber to avoid evaporation.
3.8 Rinse three times with PBS, 5 min each wash.
3.9 Additional staining with fluorescent nuclear stains or phalloidins can be done at this step.
3.10 Invert each coverslip onto a pre-cleaned slide with fluorescence anti-fade mounting media. Seal edges with clear polish if desired.
3.11 Store slides in the dark at 4°C.

Staining Protocol for Flow Cytometry

There are many alternative procedures that can be used for specific staining experiments. The protocol below is a general guideline for flow cytometry and should be optimized or modified for each application.

1. Aliquot 1 X 10^6 cells into 12 X 75 mm polypropylene tubes for flow cytometry.
2. For intracellular staining, cells can be fixed first to ensure stability of soluble antigens or antigens with short half-lives. We recommend a fix and perm kit from reliable manufacturers. Follow manufacturer's instructions.
3. Add the primary antibody or isotype control at the appropriate dilution to the assay tubes. Incubate according to manufacturer's instructions.
4. Rinse cells twice by centrifugation with 2-3 mL incubation buffer.
5. Decant supernatant and re-suspend the pellet in remaining volume of wash.
6. Add fluorescent secondary antibody and incubate for 20-30 minutes. General range for secondary antibodies is between 1-10 μg/mL for IgG conjugates for most applications.
7. Rinse cells twice by centrifugation with 2-3 mL incubation buffer. Centrifuge to collect cells after each wash. Decant supernatant.
8. Resuspend cells in 0.5 mL of diluent of choice to analyze on flow cytometer. Acquire data using the correct channel.

Tips and Hints

1) No signal or weak fluorescence intensity may suggest the following: (a) insufficient antibody is present for detection, (b) intracellular target was not accessible, (c) excitation sources are not aligned, (d) target protein is not present or expressed at low levels, (e) fluorochrome has faded, and/or (f) primary and secondary antibodies are not compatible.
2) High fluorescence intensity may suggest the following: (a) antibody concentration is too high, (b) excess antibody was not washed away efficiently, and/or (c) blocking was inadequate. Increase antibody dilution and washes.

CF™-labeled antibodies can also be used for staining histological sections from paraffin-embedded or frozen tissues.

References

1. Donaldson, J.G. Immunofluorescence staining. (2001) Curr Protoc Cell Biol. Chapter 4: Unit 4.3.
2. Blose, S.H. and Feramisco, J.R. (1983) Fluorescent methods in the analysis of cell structure. Cold Spring Harbour Laboratory.

Useful websites:
www.chroma.com

Related Products

Cat.#	Product Name	Unit Size
40061-T	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO, Trial Size (15-20 tests)	25 uL
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium with DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant	15 mL
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22002	Tween®-20	50 mL

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable fluorescent CF™ dyes, including secondary antibodies and Mix-n-Stain™ antibody labeling kits, and R-PE dye conjugates. 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.