コスモ・バイオ株式会社

Biotium
Glowing Products for Science™

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Product Information

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CF™488A Conjugated Antibodies

Catalog No.	Unit Size	Product Description
20010	0.5 mL	CF488A Goat Anti-Mouse IgG (H+L)
20010-1	50 uL	
20011	0.25 mL	CF488A Goat Anti-Mouse IgG(H+L) F(ab')2 fragment, 2 mg/mL
20012	0.5 mL	CF488A Goat Anti-Rabbit IgG(H+L)
20012-1	50 uL	
20013	0.25 mL	CF488A Goat Anti-Rabbit IgG(H+L) F(ab')2 fragment
20014	0.5 mL	CF488A Donkey Anti-Mouse IgG(H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Rabbit, Sheep, and Syrian Hamster)
20014-1	50 uL	
20015	0.5 mL	CF488A Donkey Anti-Rabbit IgG(H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Mouse, Rat, Sheep, and Syrian Hamster)
20015-1	50 uL	
20016	0.5 mL	CF488A Donkey Anti-Goat IgG(H+L), highly cross-adsorbed (min X Chicken, Guinea Pig, Horse, Human, Mouse , Rabbit, Rat, and Syrian Hamster)
20016-1	50 uL	
20017	0.5 mL	CF488A Goat Anti-Guinea Pig IgG(H+L)
20017-1	50 uL	
20018	0.5 mL	CF488A Goat Anti-Mouse IgG(H+L), highly cross-adsorbed (min X Bovine, Horse, Human, Rabbit, and Swine)
20018-1	50 uL	
20019	0.5 mL	CF488A Goat Anti-Rabbit IgG(H+L), highly cross-adsorbed (min X Human, Mouse, and Rat)
20019-1	50 uL	
20020	0.5 mL	CF488A Goat Anti-Chicken IgY(H+L), highly cross-adsorbed (min X Bovine, Goat, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, Sheep, and Syrian Hamster)
20020-1	50 uL	
20021	0.5 mL	CF488A Rabbit Anti-Goat IgG(H+L), highly cross-adsorbed (min X Human)
20021-1	50 uL	
20022	0.5 mL	CF488A Goat Anti-Human IgG(H+L), highly cross-adsorbed (min X Bovine, Horse, and Mouse)
20022-1	50 uL	
20023	0.25 mL	CF488A Goat Anti-Rat IgG(H+L), highly cross-adsorbed (min X Bovine, Horse, Human, and Rabbit)
20023-1	0.5 mL	
20024	50 uL	CF488A Donkey Anti-Sheep IgG(H+L), highly cross-adsorbed (min X Chicken, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, and Syrian Hamster)
20024-1	0.5 mL	
20025	50 uL	CF488A Rabbit Anti-Rat IgG(H+L), highly cross-adsorbed (min X Human)
20025-1	0.25 mL	
20026	0.5 mL	CF488A Rabbit Anti-Mouse IgG(H+L), highly cross-adsorbed (Min X Human)
20026-1	50 uL	
20027	0.25 mL	CF488A Donkey Anti-Rat IgG(H+L), highly cross-adsorbed (Min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Mouse, Rabbit, Sheep, and Syrian Hamster)
20027-1	0.5 mL	

Catalog No.	Unit Size	Product Description
20028	50 uL	CF488A Goat Anti-Swine IgG(H+L)
20028-1	0.25 mL	
20029	0.5 mL	CF488A Chicken Anti-Mouse IgG(H+L), highly cross-adsorbed (min X Human, Rabbit)
20029-1	50 uL	
20056	0.5 mL	CF488A Chicken Anti-Rabbit IgG(H+L), highly cross-adsorbed (min X Human, Mouse)
20056-1	50 uL	
20057	0.5 mL	CF488A Chicken Anti-Goat IgG(H+L), highly cross-adsorbed (min X Human, Mouse, and Rabbit)
20057-1	50 uL	
20071	0.5 mL	CF488A Rabbit Anti-Human IgG(H+L), highly cross-adsorbed (min X Mouse)
20071-1	50 uL	
20074	0.5 mL	CF488A Donkey Anti-Human IgG(H+L), highly cross-adsorbed (min X Bovine, Chicken, Guinea Pig, Goat, Horse, Mouse , Rabbit, Rat, Sheep, and Syrian Hamster)
20074-1	50 uL	
20079	0.5 mL	CF488A Rabbit Anti-Chicken IgY(H+L)
20079-1	50 uL	
20166	0.5 mL	CF488A Donkey Anti-Chicken IgY (IgG) (H+L), highly cross-adsorbed (min X Bovine, Goat, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, Sheep, and Syrian Hamster)
20166-1	50 uL	
20169	0.5 mL	CF488A Donkey Anti-Guinea Pig IgG (H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Horse, Human, Mouse, Rabbit, Sheep, and Syrian Hamster)
20169-1	50 uL	
20172	0.5 mL	CF488A Rabbit Anti-Sheep IgG (H+L), highly cross-adsorbed (min X Human)
20172-1	50 uL	
20208	0.5 mL	CF488A Chicken Anti-Mouse IgG (H+L)
20208-1	50 uL	
20209	0.5 mL	CF488A Chicken Anti-Rabbit IgG (H+L)
20209-1	50 uL	
20225	0.5 mL	CF488A Chicken Anti-Goat IgG (H+L)
20225-1	50 uL	

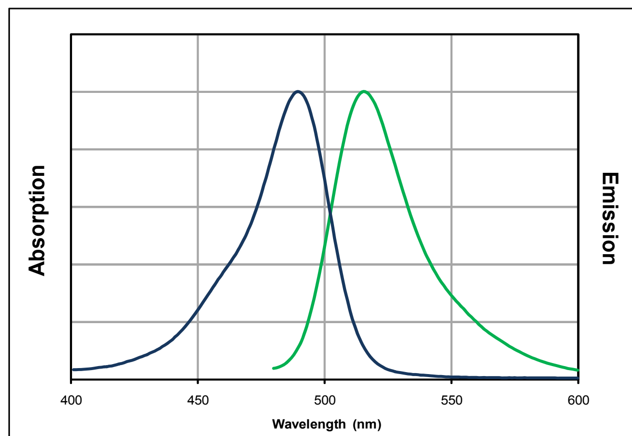
Concentration:
2 mg/mL in pH~7.4 PBS containing 50% glycerol, 2 mg/ml bovine serum albumin (IgG-free and protease-free) and 0.05% sodium azide.

Color and Form:
Yellow solution.

Storage and Handling
Product is stable for about 6 months at -20°C as an undiluted liquid. Storage of the antibody for more than a day at final working dilution is not recommended. Protect from light.

Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 490/515 nm (in pH 7.4 PBS buffer)
CF™488A is spectrally similar to Alexa Fluor® 488, DyLight® 488, Cy™ 2 and FITC.

Absorption/Emission Spectra of CF™488A Conjugates



Product Description

CF™488A antibodies are affinity-purified antibodies labeled with a green fluorescent dye, CF™488A, one of an outstanding series of CF dyes developed by Biotium. CF™ dyes are superior to both Alexa Fluor® dyes and Cy™ dyes for antibody labeling by having combined advantages in brightness, photostability, specificity and novel features ideal for in vivo imaging.

CF™488A is exceptionally bright and its fluorescence is insensitive to pH. Moreover, CF™488A is far more photostable than FITC or fluorescein. Because the wavelengths of CF™488A are slightly shorter than those of Alexa Fluor® 488, CF™488A is a better choice for instruments that use 470 nm blue light excitation and/or a fluorescence detection window that centers at a relatively shorter wavelength. For example, if your detection window is from 510 to 540 nm or centers at an even shorter wavelength, CF™488A would be a superior choice. The shorter wavelength of CF™488A offers the advantage of less fluorescence "spill-over" in the red channel in multi-color detection applications.

Another advantage CF™488A labeled antibodies is their unrivaled specificity. Like our other CF™ dyes, CF™488A is less charged than Alexa Fluor® 488. As a result, CF™488A conjugates result in better signal to noise ratio in demanding applications, such as tissue staining or certain cell staining where highly negatively charged Alexa Fluor® dyes tend to give nonspecific staining

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).

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 precleaned slide with mounting media, preferably one with an anti-fade preservative. 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.

Related Products

A full selection of secondary antibodies, antibody labeling kits, bioconjugates of phalloidin, annexin V and a-bungarotoxin are available for many of our CF™ dyes. Please visit the Biotium website at www.biotium.com for details.

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