Biotium
Glowing Products for Science™

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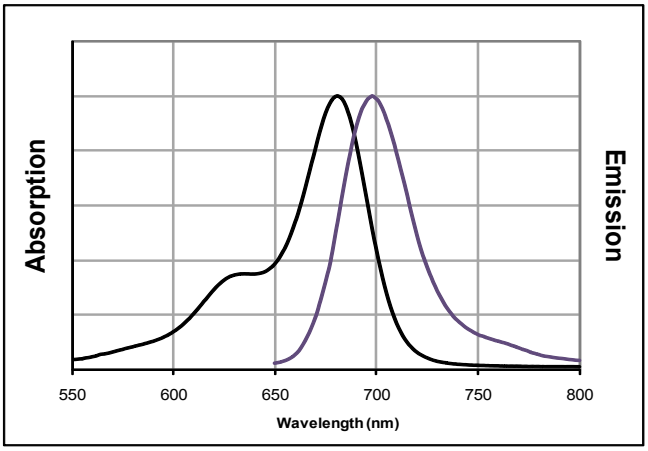
Product Information

CF™680 Conjugated Antibodies

Catalog No.	Unit Size	Product Description
20063	0.25 mL	Goat Anti-Mouse IgG (H+L) F(ab')2 fragment
20064	0.25 mL	Goat Anti-Rabbit IgG(H+L) F(ab')2 fragment
20060	0.5 mL	Donkey Anti-Goat IgG (H+L), highly cross-adsorbed (min X Chicken, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, and Syrian Hamster)
20060-1	50 uL	
20065	0.5 mL	Goat Anti-Mouse IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, Human, Rabbit, and Swine)
20065-1	50 uL	
20067	0.5 mL	Goat Anti-Rabbit IgG (H+L), highly cross-adsorbed (min X Human, Mouse, and Rat)
20067-1	50 uL	
20068	0.5 mL	Rabbit Anti-Goat IgG (H+L), highly cross-adsorbed (min X Human)
20068-1	50 uL	
20287	0.5 mL	Goat Anti-Human IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, and Mouse)
20287-1	50 uL	
20069	0.5 mL	Goat Anti-Rat IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, Human, and Rabbit)
20069-1	50 uL	
20062	0.5 mL	Donkey Anti-Sheep IgG (H+L), highly cross-adsorbed (min X Chicken, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, and Syrian Hamster)
20062-1	50 uL	
20061	0.5 mL	Rabbit Anti-Mouse IgG (H+L), highly cross-adsorbed (Min X Human)
20061-1	50 uL	
20278	0.5 mL	Donkey Anti-Human IgG (H+L), highly cross-adsorbed (min X Bovine, Chicken, Guinea Pig, Goat, Horse, Mouse , Rabbit, Rat, Sheep, and Syrian Hamster)
20278-1	50 uL	
20241	0.5 mL	Donkey Anti-Guinea Pig IgG (H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Horse, Human, Mouse, Rabbit, Sheep, and Syrian Hamster)
20241-1	50 uL	
20243	0.5 mL	Rabbit anti-Guinea Pig (H+L)
20243-1	50 uL	
20253	0.25 mL	Goat anti-mouse IgG1 (γ1)
20263	0.25 mL	Goat anti-mouse IgG2a (γ2a)
20273	0.25 mL	Goat anti-mouse IgG2b (γ2b)
20219	0.1 mL	Monoclonal mouse anti-GFP IgG

Absorption/Emission Spectra of CF680 Conjugated Antibodies



Concentration:
Secondary antibodies and mouse monoclonal anti-biotin: 2 mg/mL
Mouse monoclonal anti-GFP, anti-6X His, and anti-HA antibodies: 1 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: blue solution.

Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 681/698 nm (in pH 7.4 PBS buffer)
CF™680 is spectrally similar to Cy™5.5, Alexa Fluor® 680 and DyLight™ 680.

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.

Product Description
CF™680 antibodies are affinity-purified antibodies labeled with a near IR fluorescent dye CF™680, 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.

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General Protocols for Using CF™-labeled IgG 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.

Useful websites:

www.chroma.com

Other Related Products

You may also be interested in the following related products:

CF Dye Product	Application	Unit Size
NHS esters and maleimide	Labeling antibodies and other biomolecules	1 μmole
CF dye aminooxy and hydrazide	Labelng oligonucleotides and other biomolecules	1 mg
CF dye protein labeling kits	Labeling antibodies and other biomolecules	3 labelings
Streptavidin Conjugates	Microscopy, flow cytometry and Western blotting	1 mg
Annexin V Conjugates	Labeling apoptotic cells for microscopy or flow cytometry	0.5 mL (50 ug/mL)
Phalloidin Conjugates	Microscopy	300 U
α-Bungarotoxin	Labeling Ach-R for microscopy	0.5 mg

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