Biotium
Glowing Products for Science™

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Product Information

Revised: April 19, 2011

CF™633 Conjugated Antibodies

Catalog No.	Unit Size	Product Description
20120	0.5 mL	Goat Anti-Mouse IgG (H+L)
20120-1	50 uL	
20130	0.25 mL	Goat Anti-Mouse IgG (H+L) F(ab')2 fragment
20122	0.5 mL	Goat Anti-Rabbit IgG (H+L)
20122-1	50 uL	
20131	0.25 mL	Goat Anti-Rabbit IgG(H+L) F(ab')2 fragment
20124	0.5 mL	Donkey Anti-Mouse IgG (H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Rabbit, Sheep, and Syrian Hamster)
20124-1	50 uL	
20125	0.5 mL	Donkey Anti-Rabbit IgG (H+L), highly cross-adsorbed (min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Mouse, Rat, Sheep, and Syrian Hamster)
20125-1	50 uL	
20127	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)
20127-1	50 uL	
20129	0.5 mL	Goat Anti-Guinea Pig IgG (H+L)
20129-1	50 uL	
20121	0.5 mL	Goat Anti-Mouse IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, Human, Rabbit, and Swine)
20121-1	50 uL	
20123	0.5 mL	Goat Anti-Rabbit IgG (H+L), highly cross-adsorbed (min X Human, Mouse, and Rat)
20123-1	50 uL	
20126	0.5 mL	Goat Anti-Chicken IgY (H+L), highly cross-adsorbed (min X Bovine, Goat, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, Sheep, and Syrian Hamster)
20126-1	50 uL	
20128	0.5 mL	Rabbit Anti-Goat IgG (H+L), highly cross-adsorbed (min X Human)
20128-1	50 uL	
20132	0.5 mL	Goat Anti-Human IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, and Mouse)
20132-1	50 uL	
20133	0.25 mL	Goat Anti-Rat IgG (H+L), highly cross-adsorbed (min X Bovine, Horse, Human, and Rabbit)
20133-1	0.5 mL	
20134	50 uL	Donkey Anti-Sheep IgG (H+L), highly cross-adsorbed (min X Chicken, Guinea Pig, Horse, Human, Mouse, Rabbit, Rat, and Syrian Hamster)
20134-1	0.5 mL	
20135	50 uL	Rabbit Anti-Rat IgG (H+L), highly cross-adsorbed (min X Human)
20135-1	0.25 mL	
20136	0.5 mL	Rabbit Anti-Mouse IgG (H+L), highly cross-adsorbed (Min X Human)
20136-1	50 uL	
20137	0.25 mL	Donkey Anti-Rat IgG (H+L), highly cross-adsorbed (Min X Bovine, Chicken, Goat, Guinea Pig, Horse, Human, Mouse, Rabbit, Sheep, and Syrian Hamster)
20137-1	0.5 mL	
20138	50 uL	Goat Anti-Swine IgG (H+L)
20138-1	0.25 mL	
20139	0.5 mL	Chicken Anti-Mouse IgG (H+L), highly cross-adsorbed (min X Human, Rabbit)
20139-1	50 uL	

Catalog No.	Unit Size	Product Description
20058	0.5 mL	Chicken Anti-Rabbit IgG (H+L), highly cross-adsorbed (min X Human, Mouse)
20058-1	50 uL	
20059	0.5 mL	Chicken Anti-Goat IgG (H+L), highly cross-adsorbed (min X Human, Mouse, and Rabbit)
20059-1	50 uL	
20066	0.5 mL	Rabbit Anti-Human IgG (H+L), highly cross-adsorbed (min X Mouse)
20066-1	50 uL	
20076	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)
20076-1	50 uL	
20165	0.5 mL	Rabbit Anti-Chicken IgY (H+L)
20165	50 uL	
20168	0.5 mL	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)
20168-1	50 uL	
20171	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)
20171-1	50 uL	
20174	0.5 mL	Rabbit Anti-Sheep IgG (H+L), highly cross-adsorbed (min X Human)
20174-1	50 uL	
20222	0.5 mL	Chicken Anti-Mouse IgG (H+L)
20222-1	50 uL	
20224	0.5 mL	Chicken Anti-Rabbit IgG (H+L)
20224-1	50 uL	
20227	0.5 mL	Chicken Anti-Goat IgG (H+L)
20227-1	50 uL	
20250	0.25 mL	Goat anti-mouse IgG1 (γ1)
20260	0.25 mL	Goat anti-mouse IgG2a (γ2a)
20270	0.25 mL	Goat anti-mouse IgG2b (γ2b)

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: blue solution.

Spectral Properties

$\lambda_{abs}/\lambda_{em}$ = 630/650 nm (in pH 7.4 PBS buffer)
CF™633 is spectrally similar to Alexa Fluor® 633 and DyLight™ 633.

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

Far-red fluorescent dyes offer the advantage of ultra sensitive detection because background signal due to auto-fluorescence in most biological samples is minimal in this spectral region. For many years, the cyanine dye Cy™5 has been the dye of choice for such detection. More recently, Alexa Fluor® 647 has been developed as a better alternative by having brighter fluorescence and higher photostability. Despite the improvement, Alexa Fluor® 647 still lacks sufficient photostability for many demanding applications. On the other hand, while Alexa Fluor® 633 is photostable, its fluorescence on proteins is very weak. In fact, it has been a challenging task for dye chemists to develop a far-red fluorescent dye that is both highly fluorescent and photostable for protein and nucleic acid labeling. Using new chemistry, scientists at Biotium have successfully developed CF™633 to overcome these challenges. With its absorption peak at 630 nm, CF™633 is optimally excited by the 633 nm He-Ne laser or the 635 nm red diode laser. Its emission maximum is at 650 nm, which is 15 nm shorter than that of Alexa Fluor® 647 or Cy™5. Although the detection window on most flow cytometers is centered around the emission peak wavelength of Alexa Fluor® 647 or other Cy™5-like cyanine dyes, CF™633 is still significantly brighter (see CF633 flyer). The most important advantage of CF™633, however, is its unmatched photostability. The combination of superior brightness and photostability make CF™633 the best choice for any detection system using a 633 or 635 nm laser excitation source.

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.

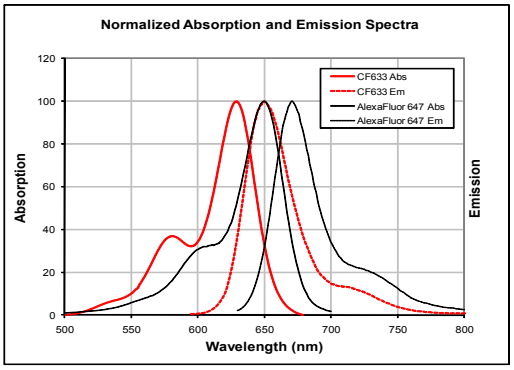
1 Aliquot 1 X 10^6 cells into 12 X 75 mm polypropylene tubes for flow cytometry.
2 For intracellular staining, cells can be fixed first to ensure stability of soluble antigens or antigens with short half-lives. We recommend a fix and perm kit from reliable manufacturers. Follow manufacturer's instructions.
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 of paraffin-embedded or frozen tissues.

Absorption/Emission Spectra of CF633 and Alexa Fluor 647



NOTE: If comparing fluorescence of CF633 on standard Cy5 or AlexaFluor 647 optimized instruments, the full advantage of CF633 may not be evident due to the spectral differences between CF633 and Cy5 or AlexaFluor 647. For best results, ensure that instrument settings are appropriate for use with CF633.

Related Products

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 our website at www.biotium.com for details.