Biotium
Glowing Products for Science™

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Product Information

CF™633 Conjugated Antibodies

Catalog No.	Product Description
20227	Chicken Anti-Goat IgG (H+L), whole antibody
20222	Chicken Anti-Mouse IgG (H+L), whole antibody
20224	Chicken Anti-Rabbit IgG (H+L), whole antibody
20168	Donkey Anti-Chicken IgG (H+L), whole antibody min X Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, and SHm
20127	Donkey Anti-Goat IgG (H+L), whole antibody Min X Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
20171	Donkey Anti-Guinea Pig IgG (H+L), whole antibody Min X Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm
20076	Donkey Anti-Human IgG (H+L), whole antibody Min X Bv, Ch, GP, Gt, Hs, Ms, Rb, Rt, Sh, SHm
20124	Donkey Anti-Mouse IgG (H+L), whole antibody Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
20125	Donkey Anti-Rabbit IgG (H+L), whole antibody Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm
20137	Donkey Anti-Rat IgG (H+L), whole antibody Min X Bv, Ch, GP, Gt, Hs, Hu, Ms, Rb, Sh, SHm
20134	Donkey Anti-Sheep IgG (H+L), whole antibody Min X Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm
20126	Goat Anti-Chicken IgG (H+L), whole antibody Min X Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm
20129	Goat Anti-Guinea Pig IgG (H+L), whole antibody
20132	Goat Anti-Human IgG (H+L), whole antibody Min X Bv, Hs, Ms
20120	Goat Anti-Mouse IgG (H+L), whole antibody
20130	$F(ab')_2$ fragment of Goat Anti-Mouse IgG (H+L)
20121	Goat Anti-Mouse IgG (H+L), whole antibody Min X Bv, Hs, Hu, Rb, Sw
20341	Goat Anti-Mouse (min x rat) IgG (H+L), whole antibody Min X Bv, Ch, Gt, GP Hs Hu Rb Rt, Sh, SHm
20122	Goat Anti-Rabbit IgG (H+L), whole antibody
20131	$F(ab')_2$ fragment of Goat Anti-Rabbit IgG (H+L)
20123	Goat Anti-Rabbit IgG (H+L), whole antibody Min X Hu, Ms, Rt
20133	Goat Anti-Rat IgG (H+L), whole antibody Min X Bv, Hs, Hu, Rb
20138	Goat Anti-Swine IgG (H+L), whole antibody
20165	Rabbit Anti-Chicken IgG (H+L), whole antibody
20128	Rabbit Anti-Goat IgG (H+L), whole antibody Min X Hu
20066	Rabbit Anti-Human IgG (H+L), whole antibody Min X Ms
20136	Rabbit Anti-Mouse IgG (H+L), whole antibody Min X Hu
20135	Rabbit Anti-Rat IgG IgG (H+L), whole antibody Min X Hu
20174	Rabbit Anti-Sheep IgG IgG (H+L), whole antibody min X Hu
20296	Bovine Anti-Goat IgG (H+L), whole antibody Min X Bv, Ch, GP, SHm, HS, Hu, Ms, Rb and Rt

Bv: bovine; Ch: chicken; Gt: goat; GP: Guinea pig; Hs: horse; Hu: human; Ms: mouse; Rb: rabbit; Sh: sheep; SHm: Syrian hamster; Sw: swine; Rt: rat

Unit Size:
Whole IgG: 50 uL or 0.5 mL (liquid format), or 1 mg (lyophilized)
$F(ab')_2$ fragment: 50 uL or 250 uL (liquid format)

Concentration:
Liquid format: 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.

Lyophilized format (after reconstitution): 2 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:
Liquid format: blue solution
Lyophilized format: blue solid

Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 630/650 nm (in pH 7.4 PBS buffer) (Figure 1)

CF™633 is spectrally similar to Alexa Fluor® 633 and DyLight® 633.

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. Liquid format antibodies contain 50% glycerol and will not freeze at -20°C.

Reconstitution (lyophilized format only): 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

Far-red fluorescent dyes offer the advantage of ultra sensitive detection because auto-fluorescence in most biological samples is low in this spectral region. However, it has been a challenge for dye chemists to develop far-red fluorescent dyes that are both highly fluorescent and photostable. Using new chemistry, scientists at Biotium have successfully developed CF™633 to overcome these challenges. CF™633 is optimally excited by the 633 nm He-Ne laser or the 635 nm red diode laser. CF™633 is significantly brighter than Cy®5, Alexa Fluor® 633, and Alexa Fluor® 647, and has unmatched photostability, making it an excellent choice for detection systems using 633 or 635 nm excitation.

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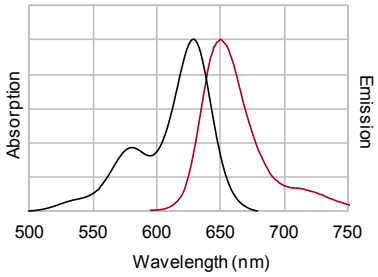
Figure 1. Absorption/Emission Spectra of CF™633 Antibody Conjugates



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.