

# Product Information

## CF™660C Conjugated Antibodies

Catalog No.	Unit Size	Product Description
20051	0.5 mL	<b>Donkey Anti-Goat IgG (H+L) whole antibody</b> (min X Chicken, Guinea Pig, Syrian Hamster, Horse, Human, Mouse, Rabbit, and Rat)
20051-1	50 uL	
20050	0.5 mL	<b>Goat Anti-Mouse IgG (H+L) whole antibody</b>
20050-1	50 uL	
20052	0.5 mL	<b>Goat Anti-Mouse IgG (H+L) whole antibody</b> (min x Human, Bovine, Horse, Rabbit, and Swine)
20052-1	50 uL	
20053	0.5 mL	<b>Goat Anti-Rabbit IgG (H+L) whole antibody</b>
20053-1	50 uL	

## CF™660R Conjugated Antibodies

Catalog No.	Unit Size	Product Description
20054	0.5 mL	<b>Goat Anti-Mouse IgG (H+L) whole antibody</b>
20054-1	50 uL	
20055	0.5 mL	<b>Goat Anti-Rabbit IgG (H+L) whole antibody</b>
20055-1	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:** blue solution.

### Spectral Properties of CF660C and CF660R

**CF660C:**  $\lambda_{abs}/\lambda_{em}$  = 667/685 nm (in pH 7.4 PBS buffer)

**CF660R:**  $\lambda_{abs}/\lambda_{em}$  = 663/682 nm (in pH 7.4 PBS buffer)

CF™660C and CF™660R are spectrally similar to Alexa Fluor® 660.

### 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™660 antibodies are affinity-purified antibodies labeled with a far-red fluorescent dye CF™ 660, one of an outstanding series of CF™ dyes developed by Biotium. CF™660C and CF™660R are two spectrally similar fluorescent dyes that emit fluorescence at about 685 nm in the borderline spectral region between far-red and near-IR (Figure 1). Although their absorption maxima are at around 660 nm, both dyes can be sufficiently excited by the 633 or 635 nm laser. When combined with other CF™ dyes of shorter wavelengths, CF™660C or CF™660R can serve as a useful long wavelength dye in multi-color detection applications. The two dyes are spectrally similar to Alexa Fluor® 660 but are far superior to the latter in performance. CF™660C is several fold brighter and significantly more photostable than Alexa Fluor® 660. CF™660R is exceptionally photostable, compared to both Alexa Fluor® 660 and CF™660C. CF™660R is also much brighter than Alexa Fluor® 660, though not as bright as CF™660C. The superior photostability and excellent brightness of CF™660R make the dye an ideal choice for confocal microscopy and other demanding applications.

A full selection of secondary antibodies, antibody labeling kits, and other bioconjugates including phalloidins, annexin V and  $\alpha$ -bungarotoxin are also available for many CF™ dyes. Please visit the Biotium website at [www.biotium.com](http://www.biotium.com) for details.

## General Protocols for Using CF™-labeled IgG Secondary Antibodies

### Recommended Dilution Range

1-10  $\mu$ g/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  $\mu$ L 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  $\mu$ g/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 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  $1 \times 10^6$  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  $\mu\text{g/mL}$  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<sup>TM</sup>-labeled antibodies can also be used for staining histological sections from paraffin-embedded or frozen tissues.

## References

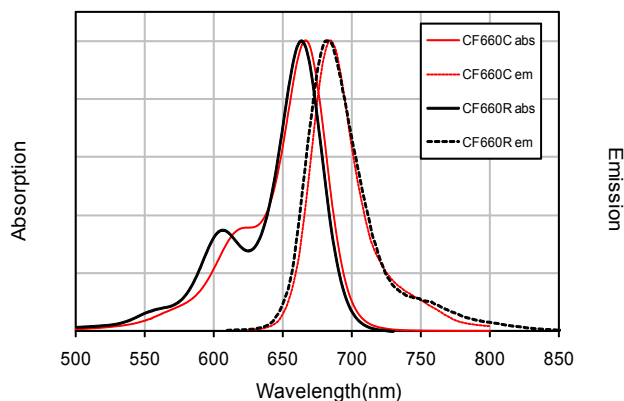
1. Donaldson, J.G. Immunofluorescence staining. (2001) Curr Protoc Cell Biol. Chapter 4: Unit 4.3.
2. Blose, S.H. and Feramisco, J.R. (1983) Fluorescent methods in the analysis of cell structure. Cold Spring Harbour Laboratory.

Useful websites:

[www.chroma.com](http://www.chroma.com)

Products are for research use only. Not for use in diagnostic or therapeutic procedures. CF<sup>TM</sup> dye technology is covered by pending US and international patents. Alexa Fluor is a registered trademark of Invitrogen.

## Absorption/Emission Spectra of CF660 Conjugated Antibodies



## 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 $\mu\text{mole}$
CF dye aminoxy and hydrazide	Labeling 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 mg/mL)
Phalloidin Conjugates	Microscopy	300 U
$\alpha$ -Bungarotoxin	Labeling Ach-R for microscopy	0.5 mg

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