Biotium

3423 Investment Blvd, Suite 8
Hayward, CA 94545
www.biotium.com

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Product Information

CF™770 Goat Anti-Rabbit IgG (H+L), highly cross-absorbed (min X Hu, Ms and Rat)

Catalog Number: 20078

Unit Size: 0.25 mL

Concentration: 2 mg/mL in pH~7.4 PBS containing 10 mg/mL bovine serum albumin (IgG-free and protease-free) and 0.01% sodium azide.

Color and Form: blue-green solution

Spectral Property

$\lambda_{abs}/\lambda_{em}$ = 770/797 nm (in pH 7.4 PBS buffer)
CF™770 is spectrally similar to IR Dye 800 and DyLight 800

Storage and Handling

Product is stable for about 6 months at 4°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™770 goat anti-rabbit IgG is an affinity-purified goat anti-rabbit IgG labeled with a green fluorescent dye CF™770, 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 (Please visit our website for details).

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.

Protocol for Western Blotting

The protocol below is a general guideline and should be optimized or modified for each application. Nitrocellulose membranes are recommended for NIR detection; however, PVDF membranes with low auto-fluorescence such as the Immobilon™-FL from Millipore are also compatible. Handle membranes by forceps only and use a pencil to label. Ink from pens may bleed or fluoresce on infrared imaging systems resulting in non-specific blotches.

1 Perform electrophoresis and transfer proteins as usual. Membranes are typically dried thoroughly before blotting.
2 Wet the membrane again in methanol (PVDF) or PBS (nitrocellulose) for 2-5 min before blocking. Block for 30-60 minutes or overnight at 4°C with gentle rocking. Blocking solutions containing BSA or dry milk may contain bovine IgG and may cause higher background. A non-mammalian serum blocking buffer or IgG-free BSA may be desired.
3 Add the primary antibody at the appropriate dilution to the blocking buffer with 0.1% Tween 20 to reduce background. Incubate with gentle rocking for 1 hr to overnight (depending on manufacturer). We strongly recommend optimization of your particular primary antibody.
4 Wash membrane 4 X 5 min with PBS containing 0.1% Tween 20 with moderate rocking.
5 Incubate membrane with a 1:10,000 to 1:50,000 dilution of the CF secondary antibody in blocking buffer + 0.1% Tween 20 for 1 hr with gentle rocking and protected from light.
6 Wash membrane 4 X 5 min with PBS + 0.1% Tween 20 with moderate rocking and protected from light.
7 Scan blot wet or dry on a infrared imaging system. Store membrane dry and protected from light.

Protocol for In-Cell Western™

The protocol below is a general guideline and should be optimized for each application. Do not allow cells to dry out at during staining and add solutions carefully down the sides of the well to avoid detaching the cells.

1 Culture cells in black-walled, clear bottom 96-well plates (80-90% confluency) and treat cells as desired.
2 Carefully aspirate or manually pipet out the media and fix cells with 200 uL per well of 3.7% paraformaldehyde/PBS for 20 min. (Add the fixative carefully down the sides of the well to avoid detaching the cells.)
3 Rinse the wells carefully with PBS, 2 X 5 min.
4 To permeabilize, wash the cells 2 X 10 min with PBS containing 0.1-0.5% Triton X-100 with gentle rocking.
5 Rinse cells once with 1X PBS.
6 Block cells for 1 hr with blocking buffer. Blocking solutions containing BSA or dry milk may contain bovine IgG and may cause higher background. A non-mammalian serum blocking buffer or IgG-free BSA may be desired.
7 Dilute primary antibody in blocking buffer + 0.1% Tween 20 to reduce background. Control wells that do not contain primary antibody (secondary antibody only) can be used to correct for background staining if desired. Incubate wells with primary antibody for 2 hr to overnight at 4°C with gentle rocking. Add 50 - 100 uL of antibody solution to each well for sufficient coverage.
8 Wash the plate with 200 uL/well of PBS + 0.1% Tween 20, 4 x 5 min with gentle rocking.
9 Incubate cells with a 1:500 to 1:2,000 dilution of the CF conjugated secondary antibody for 1 hr with gently shaking and protected from light. Add 50 - 100 uL of antibody solution to each well for sufficient coverage.
10 Wash the plate with 200 uL/well PBS + 0.1% Tween 20, 4 x 5 min with gentle rocking and protected from light.
11 After the final wash, remove all excess solution and scan plate immediately or dry completely before scanning.

Tips and Hints:

1) No signal or weak fluorescence intensity may suggest the following: (a) insufficient antibody is present for detection, (b) target protein is not accessible, not present or expressed at low levels, (c) fluorochrome has faded, (d) primary and secondary antibodies are not compatible, (e) washing was too stringent, and/or (f) excitation source or detection is not compatible. Load more lysate, increase antibody concentration(s), reduce washing, and/or check instrument settings.

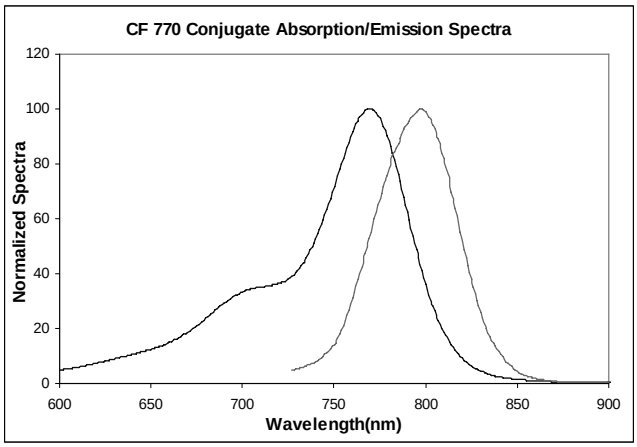
2) High fluorescence intensity may suggest the following: (a) antibody concentration is too high, (b) excess antibody was not washed away efficiently, and/or (c) blocking was inadequate. Increase antibody dilution and washes.

3) For 2-color detection, our CF680 and CF770 highly cross-adsorbed antibodies are recommended to minimize cross-reactivity and non-specific background.

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.

Absorption/Emission Spectra of CF 770 Conjugated Antibodies



*CF™ dye technology is covered by pending US and international patents.
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Other Related Products

You may also be interested in the following related products:

Table 1. List of CF Dye Product Categories*

Product Type	Application	Unit Size	Unit Price($)
CF dye NHS esters	Labeling antibodies and other biomolecules	1umole	210.00
VivoBrite near-IR CF dye labeling kit for small animal in vivo imaging	Labeling antibodies for in vivo imaging	3 labelings	395.00
CF dye goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye goat anti-guinea pig IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye F(ab')2 fragment of goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	105.00
CF dye F(ab')2 fragment of goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	105.00
Near-IR CF dye goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	150.00
Near-IR CF dye goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	150.00
Near-IR CF dye goat-anti-mouse IgG (H+L), highly cross-adsorbed, 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	160.00
Near-IR CF dye goat-anti-rabbit IgG (H+L), highly cross-adsorbed, 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	160.00
CF dye annexin V conjugates, 50 ug/mL	Apoptosis	0.5 mL	210.00
CF dye phalloidin conjugates	F-actin staining	300 U	295.00

*For a complete list and descriptions of individual products, please visit biotium website: www.biotium.com