Revised: January 21, 2019

Product Information

CF® Dye Conjugated Monoclonal Mouse Anti-GFP Antibodies

Catalog No.	Product Description
20215	CF®488A Monoclonal Mouse Anti-GFP
20480	CF®568 Monoclonal Mouse Anti-GFP
20216	CF®594 Monoclonal Mouse Anti-GFP
20217	CF®633 Monoclonal Mouse Anti-GFP
20218	CF®640R Monoclonal Mouse Anti-GFP
20481	CF®660R Monoclonal Mouse Anti-GFP
20219	CF®680 Monoclonal Mouse Anti-GFP
20482	CF®680R Monoclonal Mouse Anti-GFP
20220	CF®770 Monoclonal Mouse Anti-GFP

Unit size: 0.1 mL

Concentration: 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.

Storage and Handling

Product is stable for at least 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.

Spectral Properties

$\lambda_{abs}/\lambda_{em}$ maxima in pH 7.4 PBS buffer

Conjugate	Abs (nm)	Em (nm)
CF®488A	490	515
CF®568	562	583
CF®594	593	614
CF®633	630	650
CF®640R	642	662
CF®660R	663	682
CF®680	681	698
CF®680R	680	701
CF®770	770	797

Product Description

Monoclonal mouse anti-GFP antibodies (clone 9F9.F9, IgG1κ) are raised against full length recombinant green fluorescent protein and are conjugated to Biotium's bright and photostable CF® dyes.

General Protocols for Using CF® Dye Conjugated Antibodies

Recommended Dilution Range

Working concentrations of IgG conjugates range from 1-10 ug/mL for most applications. Optimal concentration 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

Note: exposure to solvents (such as alcohol fixation or during paraffin embedding) will destroy green fluorescent protein's intrinsic fluorescence, however, anti-GFP antibodies will react with GFP after solvent exposure.

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 uL 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. Working concentrations of IgG conjugates range from 1-10 ug/mL for most applications. We recommend including samples without primary antibody as a control for background staining. 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 methods for cell staining. 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, fix and permeabilize cells according to standard protocols. Biotium also offers a fixation/permeabilization kit for flow cytometry (see related products).
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. Working concentrations of IgG conjugates range from 1-10 μg/mL 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.

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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.
3. www.chroma.com

Related Products

Cat. No.	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 streptavidin and other bioconjugates, secondary antibodies, Mix-n-Stain™ antibody labeling kits, and R-PE 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.