Revised: January 23, 2019

Product Information

Drop-n-Stain™ Secondary Antibodies

Catalog No.	Product Description
20950	CF®488A Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Rt, Sh, SHm
20951	CF®594 Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Rt, Sh, SHm
20952	CF®488A Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
20953	CF®594 Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
20954	CF®488A Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Hu, Ms, Rt
20955	CF®594 Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Hu, Ms, Rt
20956	CF®488A Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Hs, Hu, Rb, Sw
20957	CF®594 Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Hs, Hu, Rb, Sw
20962	CF®640R Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Rt, Sh, SHm
20963	CF®640R Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
20964	CF®640R Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Hu, Ms, Rt
20965	CF®640R Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Hs, Hu, Rb, Sw
20966	CF®543 Donkey Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Rt, Sh, SHm
20967	CF®543 Donkey Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm
20968	CF®543 Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed Min X Hu, Ms, Rt
20969	CF®543 Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed Min X Bv, Hs, Hu, Rb, Sw

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: 5 mL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties

CF®488A:
$\lambda_{abs}/\lambda_{em}$ = 490/515 nm (in pH 7.4 PBS buffer)
Spectrally similar to Alexa Fluor® 488, DyLight® 488, Cy® 2 and FITC.

CF®543:
$\lambda_{abs}/\lambda_{em}$ = 541/560 nm (in pH 7.4 PBS buffer)
Spectrally similar to Alexa Fluor® 546, Tetramethylrhodamine (TAMRA)

CF®594:
$\lambda_{abs}/\lambda_{em}$ = 593/614 nm (in pH 7.4 PBS buffer)
Spectrally similar to Alexa Fluor® 594, Cy® 3.5, Texas Red®, and DyLight® 594.

CF®640R:
$\lambda_{abs}/\lambda_{em}$ = 642/662 nm (in pH 7.4 PBS buffer)
Spectrally similar to Cy®5, Alexa Fluor® 647 and ATTO™647N.

Product Description

Drop-n-Stain™ Secondary Antibodies are provided in a convenient dropper bottle for easy dispensing without pipetting. Drop-n-Stain™ secondaries feature affinity-purified, highly cross-adsorbed secondary antibodies conjugated to Biotium's CF® dyes. CF® dyes are superior to Alexa Fluor®, DyLight®, and Cy® dyes for antibody labeling by having combined advantages in brightness, photostability, and specificity. For more information about CF® dyes, download our CF® Dye Selection Guide at www.biotium.com.

Recommended dilutions

For fluorescence microscopy: add two drops of Drop-n-Stain™ Secondary Antibody to 1 mL of the antibody diluent of your choice.

For flow cytometry: add one drop of Drop-n-Stain™ Secondary Antibody per flow tube containing 10^6 cells.

Protocols

There are many variations of antibody detection methods. These protocols are intended as general guidelines and should be optimized for best results.

Immunofluorescence Staining for Microscopy

1. Fix cells or unfixed frozen tissue sections with 4% paraformaldehyde/PBS, 15 minutes at room temperature.

   Note: other fixatives or fixation temperatures may be optimal for specific antibodies or antigens. Antigen retrieval methods may be recommended for staining paraffin-embedded tissue section with certain antibodies. See antibody manufacturer's instructions.

2. Rinse twice with PBS to remove traces of fixative.
3. Block and permeabilize cells in PBS/0.1% Triton X-100 containing the blocking agent of your choice (e.g., BSA, normal goat serum, or fish gelatin at 1-5%).
4. Dilute primary antibody in blocking/permeabilization buffer at the recommended concentration. Add diluted antibody to cover cells completely.

   Note: For cells on coverslips or tissue sections on slides, add a drop (50-100 uL) of staining solution and overlay with a piece of Parafilm® to spread solution evenly over the specimen. Keep samples in a humidified chamber to avoid evaporation.

5. Incubate 1-2 hours at room temperature or overnight at 4°C.
6. Rinse twice with PBS, then wash 3 x 5 minutes with PBS.

7. Prepare secondary staining solution by adding two drops of Drop-n-Stain™ Secondary Antibody to 1 mL dilution buffer. Counterstains or phalloidin conjugates also can be added at this step. Cover cells with secondary staining solution and incubate for 30 minutes to 2 hours at room temperature. Protect samples from light during this and subsequent steps.
8. Rinse twice with PBS, then wash 3 x 5 minutes with PBS.
9. Mount samples in fluorescence antifade mounting media. Store samples in the dark at 4°C until ready to image.

Staining for Flow Cytometry

1. Detach adherent cells from substrate prior to staining.
2. Adjust cell density to 10^7 cells per mL and aliquot 100 uL per 12 X 75 mm polypropylene flow cytometry tubes for 10^6 cells per tube.
3. For surface staining of unfixed cells, proceed to step 4 and perform all antibody incubations and washes at 4°C. For intracellular staining, fix and permeabilize cells according to your standard protocols, or use a commercial fixation/permeabilization kit (see catalog. no. 23006). For fixed cells, perform staining and washing steps at room temperature.
4. Add the recommended amount of primary antibody or isotype control to each tube in incubation buffer for surface staining or permeabilization buffer for intracellular staining. Mix and incubate 30 minutes.
5. Wash cells twice with 2-3 mL of your preferred wash buffer. Centrifuge at 350 x*g* to collect cells after each wash. Decant supernatant.
6. Resuspend the pellet in the residual buffer remaining after the last wash.
7. Add one drop of Drop-n-Stain™ Secondary Antibody to each tube. Mix and incubate 30 minutes. Protect samples from light during this and subsequent steps.
8. Wash cells as in step 5.
9. Resuspend cells in 0.5 mL wash buffer and perform flow cytometry.

Related Products

Cat. No.	Product Name
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
23008	Drop-n-Stain EverBrite™ Mounting Medium
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
23007	TrueBlack™ Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System
40061	RedDot™2 Far Red Nuclear Counterstain
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
22003	Mini-Cell Scrapers
23006	Flow Cytometry Fixation/Permeabilization Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable fluorescent CF® dyes, including Mix-n-Stain™ antibody labeling kits, primary antibody conjugates, streptavidin, phalloidin, and other bioconjugates, as well as conjugates of biotin, HRP, AP, R-PE, APC and PerCP.