Biotium
Glowing Products for Science™

| Unit Size | |
|---|---|
| XXXXX-1 | 50uL (liquid format) |
| XXXXX | 0.5mL (liquid format) |
| XXXXX-1MG | 1mg (lyophilized) |

コスモ・バイオ株式会社

※本プロトコールは参考用の資料になります。商品ご購入の際は必ず商品に添付されている資料をご参照ください。

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# Product Information

## CF™543 Conjugated Antibodies

| Catalog No. | Product Description |
|---|---|
| 20313 | Bovine Anti-Goat IgG (H+L), whole antibody<br>Min X Bv, Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm |
| 20310 | Donkey Anti-Chicken IgG (H+L), whole antibody<br>Min X Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm |
| 20314 | Donkey Anti-Goat IgG (H+L), whole antibody<br>Min X Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm |
| 20316 | Donkey Anti-Guinea Pig IgG (H+L), whole antibody<br>Min X Bv, Ch, Gt, Hs, Hu, Ms, Rb, Sh, SHm |
| 20318 | Donkey Anti-Human IgG (H+L), whole antibody<br>Min X Bv, Ch, GP, Gt, Hs, Ms, Rb, Rt, Sh, SHm |
| 20305 | Donkey Anti-Mouse IgG (H+L), whole antibody<br>Min X Bv, Ch, Gt, GP, Hs, Hu, Rb, Sh, SHm |
| 20308 | Donkey Anti-Rabbit IgG (H+L), whole antibody<br>Min X Bv, Ch, Gt, GP, Hs, Hu, Ms, Sh, SHm |
| 20320 | Donkey Anti-Rat IgG (H+L), whole antibody<br>Min X Bv, Ch, GP, Gt, Hs, Hu, Ms, Rb, Sh, SHm |
| 20322 | Donkey Anti-Sheep IgG (H+L), whole antibody<br>Min X Ch, GP, Hs, Hu, Ms, Rb, Rt, SHm |
| 20311 | Goat Anti-Chicken IgG (H+L), whole antibody<br>Min X Bv, Gt, GP, Hs, Hu, Ms, Rb, Rt, Sh, SHm |
| 20317 | Goat Anti-Guinea Pig IgG (H+L), whole antibody |
| 20319 | Goat Anti-Human IgG (H+L), whole antibody<br>Min X Bv, Hs, Ms |
| 20306 | Goat Anti-Mouse IgG (H+L), whole antibody |
| 20329 | $F(ab')_2$ fragment of Goat Anti-Mouse IgG (H+L) |
| 20299 | Goat Anti-Mouse IgG (H+L), whole antibody<br>Min X Bv, Hs, Hu, Rb, Sw |
| 20328 | Goat Anti-Mouse (min x rat) IgG (H+L), whole antibody<br>Min X Bv, Ch, Gt, GP Hs Hu Rb Rt, Sh, SHm |
| 20309 | Goat Anti-Rabbit IgG (H+L), whole antibody |
| 20330 | $F(ab')_2$ fragment of Goat Anti-Rabbit IgG (H+L) |
| 20300 | Goat Anti-Rabbit IgG (H+L), whole antibody<br>Min X Hu, Ms, Rt |
| 20321 | Goat Anti-Rat IgG (H+L), whole antibody<br>Min X Bv, Hs, Hu, Rb |
| 20324 | Goat Anti-Swine IgG (H+L), whole antibody |
| 20312 | Rabbit Anti-Chicken IgG (H+L), whole antibody |
| 20315 | Rabbit Anti-Goat IgG (H+L), whole antibody |
| 20307 | Rabbit Anti-Mouse IgG (H+L), whole antibody<br>Min X Hu |
| 20323 | Rabbit Anti-Sheep IgG (H+L), whole antibody<br>Min X Hu |

| Catalog No. | Product Description |
|---|---|
| 20333 | Chicken Anti-Goat IgG (H+L), whole antibody |
| 20334 | Chicken Anti-Mouse IgG (H+L), whole antibody |
| 20335 | Chicken Anti-Rabbit IgG (H+L), whole antibody |
| 20336 | Rabbit Anti-Guniea Pig IgG (H+L), whole antibody |

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 0.25 mL (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: pink solution
Lyophilized format: pink solid

### Spectral Properties
$\lambda_{abs}/\lambda_{em}$ = 541/560 nm (in pH 7.4 PBS buffer).

CF543 is spectrally similar to tetramethylrhodamine (TAMRA) and Alexa Fluor® 546.

### 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.

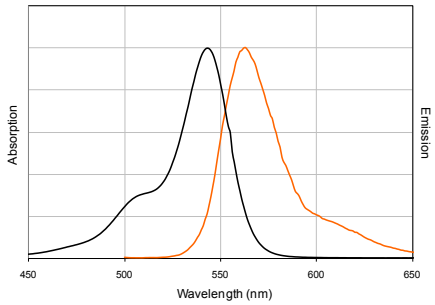
Reconstitution (lyophilized format only): add 0.5 mL $dH_2O$ and mix gently to dissolve. Store at -20°C, protected from light. Aliquot to avoid freeze-thaw cycles. Alternatively, glycerol can be added to the antibody so that it will not freeze at -20°C: add 0.25 mL $dH_2O$ to the lyophilized antibody 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

CF™543 is an orange fluorescent dye with absorption and emission maxima at 541 and 560 nm, respectively. The dye is based on a new class of rhodamine dyes invented by Biotium scientists. Unlike traditional rhodamine dyes, CF™543 is not only bright and photostable but also highly water-soluble. As a result, CF™543 antibody conjugates retain excellent solubility and yield the brightest fluorescence among antibody conjugates prepared from spectrally similar dyes. For example, CF™543 labeled goat anti-mouse IgG is significantly brighter than the antibody labeled with Alexa Fluor® 546 at similar degree of labeling.

Figure 1. Absorption/Emission Spectra of CF543 Conjugated Antibodies




## 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.

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