

# Product Information

## Goat Anti-Llama IgG (H+L), Whole Antibody

| CF® Dye | Ex/Em (nm) | Cat. No.    | Size               |
|---------|------------|-------------|--------------------|
| CF®405S | 411/431 nm | 20844-50uL  | 50 uL              |
|         |            | 20844-500uL | 500 uL             |
|         |            | 20844-1mg   | 1 mg (lyophilized) |
| CF®488A | 490/516 nm | 20845-50uL  | 50 uL              |
|         |            | 20845-500uL | 500 uL             |
|         |            | 20845-1mg   | 1 mg (lyophilized) |
| CF®543  | 543/563 nm | 20846-50uL  | 50 uL              |
|         |            | 20846-500uL | 500 uL             |
|         |            | 20846-1mg   | 1 mg (lyophilized) |
| CF®555  | 554/568 nm | 20847-50uL  | 50 uL              |
|         |            | 20847-500uL | 500 uL             |
|         |            | 20847-1mg   | 1 mg (lyophilized) |
| CF®568  | 562/584 nm | 20848-50uL  | 50 uL              |
|         |            | 20848-500uL | 500 uL             |
|         |            | 20848-1mg   | 1 mg (lyophilized) |
| CF®594  | 593/615 nm | 20849-50uL  | 50 uL              |
|         |            | 20849-500uL | 500 uL             |
|         |            | 20849-1mg   | 1 mg (lyophilized) |
| CF®640R | 642/663 nm | 20850-50uL  | 50 uL              |
|         |            | 20850-500uL | 500 uL             |
|         |            | 20850-1mg   | 1 mg (lyophilized) |
| CF®647  | 652/668 nm | 20851-50uL  | 50 uL              |
|         |            | 20851-500uL | 500 uL             |
|         |            | 20851-1mg   | 1 mg (lyophilized) |
| CF®660C | 667/685 nm | 20852-50uL  | 50 uL              |
|         |            | 20852-500uL | 500 uL             |
|         |            | 20852-1mg   | 1 mg (lyophilized) |
| CF®660R | 662/682 nm | 20853-50uL  | 50 uL              |
|         |            | 20853-500uL | 500 uL             |
|         |            | 20853-1mg   | 1 mg (lyophilized) |
| CF®680  | 681/698 nm | 20854-50uL  | 50 uL              |
|         |            | 20854-500uL | 500 uL             |
|         |            | 20854-1mg   | 1 mg (lyophilized) |
| CF®680R | 680/701 nm | 20855-50uL  | 50 uL              |
|         |            | 20855-500uL | 500 uL             |
|         |            | 20855-1mg   | 1 mg (lyophilized) |
| CF®750  | 755/779 nm | 20856-50uL  | 50 uL              |
|         |            | 20856-500uL | 500 uL             |
|         |            | 20856-1mg   | 1 mg (lyophilized) |
| HRP     | N/A        | 20475-100uL | 100 uL             |
|         |            | 20475-1mL   | 1 mL               |
|         |            | 20475-1mg   | 1 mg (lyophilized) |
| DNP     | N/A        | 20983-50uL  | 50 uL              |
|         |            | 20983-500uL | 500 uL             |
|         |            | 20983-1mg   | 1 mg (lyophilized) |

### 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<sub>2</sub>O 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<sub>2</sub>O 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.

#### Notes:

- Do not add sodium azide to HRP conjugates.
- Storage of the antibody for more than a day at final working dilution is not recommended.

### Concentration

#### Liquid format:

- CF® Dye conjugates: 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
- HRP conjugates: 1 mg/mL in pH ~7.4 PBS containing 50% glycerol and 15 mg/ml bovine serum albumin (IgG-free and protease-free)
- DNP conjugates: 2 mg/mL for DNP conjugates in pH ~7.4 PBS, 50% glycerol, 2 mg/mL bovine serum albumin (IgG-free and protease-free), and 0.05% azide

#### Lyophilized format (after reconstitution):

- CF® Dye conjugates: 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
- HRP conjugates: 1 mg/mL in pH ~7.4 PBS containing 10 mg/ml trehalose
- DNP conjugates: 1 mg/mL in pH ~7.4 PBS, 15 mg/mL bovine serum albumin (IgG-free and protease-free), 20 mg/mL trehalose

### Product Description

Goat anti-llama IgG (H+L), whole antibody is an affinity-purified goat antibody that recognizes llama IgG. This antibody reacts with whole llama IgG and is not recommended for detection of camelid single variable VHH domains (Nanobodies®). It is not highly-cross adsorbed against other species. Visit [www.biotium.com](http://www.biotium.com) to see our highly cross-adsorbed secondary antibodies for multiple labeling and isotype-specific secondary antibodies.

Biotium's next-generation CF® Dyes were designed to be highly water-soluble with advantages in brightness and photostability compared to other commercially available fluorescent dyes. For more information about CF® Dyes, please visit our website at [www.biotium.com](http://www.biotium.com).

### Recommended Dilution Range

- Fluorescence microscopy: 1-2 ug/mL
- Flow cytometry: 1 ug/10<sup>6</sup> cells
- Near-infrared western detection: 50-100 ng/mL

These concentrations are provided as a starting point for optimization; appropriate dilutions should be determined empirically. Generally, IgG conjugates are used in the range of 1-10 ug/mL.

For more detailed protocols for immunofluorescence staining for fluorescence microscopy, flow cytometry, or fluorescence-based western detection, please visit our website.

## Basic Protocols for Antibody-Based Detection

The following are general protocols for immunofluorescence staining with CF® Dye labeled antibodies for detection of proteins by microscopy, flow cytometry, or western blot. Please note, there are many variations of antibody detection methods. These protocols are intended as general guidelines and should be optimized for best results.

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## Immunofluorescence Staining for Microscopy

- This is a protocol for staining fixed and permeabilized cells. Staining of tissue sections may require antigen retrieval or autofluorescence quenching. See our [Tech Tip: Considerations for Immunofluorescence Staining](#) for more information.
- Conjugates of blue-fluorescent dyes like CF®405S is not as bright as other colors and are not recommended for detecting low abundance targets. Blue dyes are also challenging to use in tissue specimens, which have high autofluorescence in blue wavelengths.

### Materials required

- PBS or HBSS (buffer with Ca<sup>2+</sup>/Mg<sup>2+</sup> may be optimal for adherent cells)
- Paraformaldehyde, 4% in PBS, or methanol pre-chilled to -20°C (see notes in step 2 of this procedure)
- 1X PBS (Ca<sup>2+</sup>/Mg<sup>2+</sup>-free is acceptable)
- PBS + 2% fish gelatin + 0.1% Triton® X-100 + 0.02% sodium azide
- CF® Dye Antibody Conjugate
- Antifade mounting medium
- Coverslip sealant (for wet-mounted coverslips only)

### Workflow overview

- Fix (≤20 minutes) (optional stopping point)
- Block/permeabilize (30 minutes)
- Antibody conjugate incubation (1-2 hours or overnight)
- Washes (20-30 minutes)
- Mount (optional stopping point)
- Image slide

### Procedure

1. Rinse cells twice with PBS or HBSS to remove cell culture medium. Use the same volume for washes as you would for cell culture medium (we use 100 uL per well of a 96-well plate). For some cell types, buffer with Ca<sup>2+</sup>/Mg<sup>2+</sup> may be necessary to prevent cell rounding and detachment. Prior to fixation, we prefer to use HBSS + Ca<sup>2+</sup>/Mg<sup>2+</sup> for adherent cells.

2. Fix cells with 4% paraformaldehyde/PBS, 20 minutes at room temperature. Alternatively, fix cells in pre-chilled methanol at -20°C for 5-10 minutes.

**Note:** Different fixation methods may be optimal for your target of interest. If the optimal fixation conditions are unknown, it may be necessary to test different fixation methods for a specific target.

3. Rinse three times with PBS to remove traces of fixative.

**Note:** In our experience, cells can be stored in PBS after fixation for several weeks. We recommend using PBS with 0.02%-0.05% sodium azide as a preservative. Keep samples well-sealed or in a humidified box to avoid evaporation of buffer.

4. Block and permeabilize cells in PBS + 2% fish gelatin + 0.1% Triton® X-100.

**Note:** Other blocking reagents, such as BSA, may also be used instead of fish gelatin. When using some highly negatively charged fluorescent dyes, specialized blocking buffers such as our TrueBlack® IF Background Suppressor System may help reduce background (see Products for Immunofluorescence).

5. Dilute the antibody conjugate in fresh blocking/permeabilization buffer. The optimal concentration should be determined empirically. For fluorescence microscopy, we recommend 2-5 ug/mL as a starting point for optimization.

**Note:** You may need to perform a titration experiment to determine the optimal concentration of primary antibody.

6. Add enough diluted antibody solution to cover cells completely. We usually use 50-100 uL per well of a 96-well plate.

**Note:** For cells on coverslips, add 50-100 uL of diluted antibody solution and overlay with a piece of Parafilm® to spread solution evenly over the specimen, making sure there are no bubbles. Keep samples in a humidified chamber to avoid evaporation.

7. Incubate 1-2 hours at room temperature or overnight at 4°C (in our experience, 4°C overnight gives the best results). Protect from light.

**Note:** Other stains, such as nuclear counterstains, lectins, or phalloidin conjugates can be added together with labeled antibodies at this step.

8. Rinse cells twice with PBS, then wash 3 x 5 minutes with PBS.

**Note:** Alternatively, rinse cells twice with PBS, incubate in PBS for 30 minutes, then rinse with PBS. Cells can be left in PBS longer without negatively affecting staining.

9. Mount samples in fluorescence antifade mounting media such as EverBrite™ Mounting Medium (medium with DAPI can be used for blue nuclear counterstaining). For chambered coverglass or multi-well coverglass plates, remove all traces of buffer and add enough mounting medium to completely cover the cells.

**Note:** For coverslips, wet-set or hard-set mounting medium may be used. Follow mounting medium instructions for mounting coverslips. If wetset mounting medium is used, the edges of the coverslip must be sealed with nail polish or CoverGrip™ Coverslip Sealant (recommended) before imaging.

10. Store samples at 4°C and protected from light until ready to image. Usually, antibody-stained samples can be stored in mounting medium at 4°C for six months or longer.

## Cell Surface Staining for Flow Cytometry

### Materials required

- Live-or-Dye™ Fixable Viability Stain (Cat. No. 32002... 32018) or dead cell nucleic acid stain (optional)
- CF® Dye Antibody Conjugate
- Fixation Buffer (Cat. No. 22015) (optional)
- Flow buffer (PBS + 2% bovine serum or BSA + 0.02% sodium azide)
- Flow cytometry tubes (12 x 75 mm polypropylene tubes)

### Workflow overview

- Aliquot cells to flow tubes
- Dead cell staining (optional)
- Antibody incubation (30 minutes)
- Wash and centrifuge (2 x 5 minutes)
- Fixation followed by wash (optional)
- Analyze by flow cytometry

### Procedure

1. Detach adherent cells from substrate by trypsinization or with a commercial non-enzymatic cell lift solution.

2. Optional: To exclude dead cells from analysis, resuspend cells in PBS or other protein-free buffer and stain cells with a fixable dead cell dye, such as our Live-or-Dye™ Fixable Viability Stains, according to the product protocol.

**Note:** If cell fixation will not be performed, a non-fixable dead cell stain, such as PI or TO Iodide (also known as TO-PRO®-1), can be added together with primary antibody. [View our full selection of non-fixable dead cell stains.](#)

3. Adjust cell density to 10<sup>7</sup> cells per mL in flow buffer.

4. Aliquot 100 uL of cell suspension per flow cytometry tube for a total of 10<sup>6</sup> cells per tube. Place tubes on ice.

5. Add antibody conjugate to tubes and vortex gently to mix. Incubate tubes on ice (or at 4°C) for 30 minutes. Protect from light.

**Note:** Antibody conjugate concentration must be optimized for different targets but 0.5-1 ug antibody per tube is a common starting concentration.

6. Wash by adding 1 mL flow buffer to each tube and pellet cells by centrifugation for 5 minutes at 350 x g.
7. Carefully remove the supernatant from the tubes and dispose into a waste container.
8. Repeat wash (steps 6-7).
9. Optional: Cells can be fixed at this step with your preferred fixative. After fixation, wash as in steps 6-7.
10. After removing the supernatant, add 500 uL flow buffer per tube.
11. Analyze by flow cytometry in the correct channel for your conjugate. Mix by gentle vortexing before loading each sample on cytometer.

**Note:** If fixation is performed in step 9, cells can be stored at 4°C, protected from light, for several days before analysis.

## Intracellular Staining for Flow Cytometry

### Materials required

- 1X PBS
- Live-or-Dye™ Fixable Viability Stain (Cat. No. 32002... 32018) (optional)
- Flow Cytometry Fixation/Permeabilization Kit (Cat. No. 23006) (optional)
- CF® Dye Antibody Conjugate
- Flow buffer (PBS + 2% bovine serum or BSA + 0.02% sodium azide)
- Flow cytometry tubes (12 x 75 mm polypropylene tubes)

### Workflow overview

- Aliquot cells to flow tubes
- Dead cell staining (optional)
- Fixation (20 minutes)
- Wash and centrifuge (5 minutes)
- Permeabilization/antibody conjugate incubation (30 minutes)
- Wash and centrifuge (2 x 5 minutes)
- Analyze by flow cytometry

### Procedure

1. Detach adherent cells from substrate by trypsinization or with a commercial non-enzymatic cell lift solution.
2. Optional: To exclude dead cells from analysis, resuspend cells in PBS and stain with a fixable dead cell dye, such as our Live-or-Dye™ Fixable Viability Stains according to the product protocol.
3. Optional: Perform antibody staining for cell surface markers (see Cell Surface Antibody Staining for Flow Cytometry).
4. Adjust cell density to 10<sup>7</sup> cells per mL in PBS.
5. Aliquot 100 uL cell suspension to each 12 x 75 mm polypropylene flow cytometry tubes for a total of 10<sup>6</sup> cells per tube.
6. Add 100 uL fixation buffer to each tube and mix by gentle vortexing. Incubate at room temperature for 20 minutes.  
**Note:** Protect tubes from light if cells are stained with a fluorescent primary antibody.
7. Add 1 mL PBS to each tube and pellet cells by centrifugation for 5 minutes at 350 x g.
8. Remove the supernatant from the tubes and dispose into a waste container.
9. Add 100 uL permeabilization buffer to each tube and mix by gentle vortexing.
10. Add antibody conjugate to the tubes and vortex gently to mix. Incubate at room temperature for 30 minutes. Protect from light.  
**Note:** Antibody conjugate concentration must be optimized for different targets but 0.5-1 ug antibody per tube is a common starting concentration.
11. Wash by adding 1 mL flow buffer to each tube. Pellet cells by centrifugation for 5 minutes at 350 x g.
12. Remove the supernatant from the tubes and repeat step 11.
13. Remove the supernatant, then add 500 uL of flow buffer per tube.
14. Analyze by flow cytometry in the correct channel for your conjugate. Mix by gentle vortexing before loading each sample on the cytometer.

**Note:** Cells can be stored at 4°C, protected from light, for several days.

## Fluorescent Western Blotting

### Materials required

- VersaBlot™ Total Protein Normalization Kit (Cat. No. 33025, 33026) (optional)
- TrueBlack® WB Blocking Buffer Kit (Cat. No. 23013) (optional) (see general considerations below)
- PBS or TBS with 0.1% Tween®-20 + 0.02% azide (see general considerations below)
- CF® Dye Antibody Conjugate

### Workflow overview

- Optional: Perform total protein prestaining
- Perform SDS-PAGE and protein transfer (~2 hours) (optional stopping point)
- Optional: Confirm protein transfer
- Blocking (30-60 minutes)
- Antibody incubation (2 hours or overnight)
- Washes (~15-30 minutes)
- Dry membrane (optional stopping point)
- Image membrane

### General considerations for fluorescent western detection

- The following protocol is designed for a typical western blot transfer setup. If using an automated or fast-transfer system, please follow that device's protocol.
- Multiplex fluorescence western detection requires an imaging system capable of detecting multiple fluorescent conjugates. For best results, use a gel imager or scanner specifically designed for imaging fluorescent blots.
- Reducing or non-reducing SDS-PAGE may be optimal for detecting different proteins. We recommend checking the literature for your target protein of interest, or comparing reducing and non-reduced samples to determine the optimal conditions.
- Far-red or near-infrared dyes are optimal for fluorescent western, because background is lower in these wavelengths. Visible fluorescent dyes can be used, but generally will have lower signal-to-noise ratio due to higher autofluorescence of proteins and blotting membranes in the visible spectrum.
- Optimal protein loading amount varies depending on detection method and target expression level, but ranges between 1-10 ug/lane for most applications.
- As a protein marker, we recommend using Peacock™ Prestained Protein Markers which have bands that range from 8 kDa to 245 kDa. The bands fluoresce in the 700 channel and are colored blue and red so you can monitor your gel electrophoresis and transfer. We recommend using 1.5-3 uL of ladder per lane for fluorescence detection (see Products for Immunofluorescence).
- Blue tracking dyes in SDS-PAGE loading buffer can fluoresce in the far-red/near-infrared spectra; loading buffer with an orange tracking dye is recommended for fluorescent western detection. Biotium offers a 4X Protein Loading Buffer with Orange Tracking Dye for fluorescent western blots (see Products for Immunofluorescence).
- We recommend using low-fluorescence PVDF for fluorescent western blot detection. Nitrocellulose membranes may also be used and in our experience have shown similar background fluorescence to low-fluorescence PVDF.
- Ponceau S Solution is not recommended for near-IR western blots due to its poor sensitivity for low loading amounts, especially on PVDF. For total protein staining, we recommend VersaBlot™ Total Protein Normalization Kits due to their exceptional linearity, ease of use, and downstream reversibility for multi-color analysis (see Products for Immunofluorescence).
- 9 cm<sup>2</sup> petri dishes hold 5-10 mL and are convenient for washing and incubating mini-blots. Alternatively, commercially available black blotting boxes for fluorescent westerns come in a variety of sizes for blots or membrane strips.
- Either phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) can be used for fluorescent western detection with similar results.
- BSA, non-fat dry milk, and fish gelatin can be used for western blot blocking and antibody dilution buffers. These blocking agents are usually used at 1-5% in PBS (or TBS) + 0.1% Tween®-20. Commercially available blocking buffers developed specifically for fluorescent western detection, such as our TrueBlack® WB Blocking Buffer, can give lower background than other blocking agents.

- It may be desirable to minimize the volume of antibody solutions used for blotting by using sealable bags or small containers. Enough solution should be used to freely move across the blot without trapping bubbles.
- For blocking and wash steps, always use plenty of liquid to cover the blot. Use 5-10 mL buffer for a mini-blot. The blot should move freely in the buffer.

#### Procedure

1. Optional: To fluorescently label total protein in your sample for transfer confirmation and western normalization, use a total protein prestaining kit, such as our VersaBlot™ Total Protein Normalization Kit, according to the kit protocol (see Products for Immunofluorescence).
2. Perform SDS-PAGE and western transfer using standard protocols.  
**Note:** After transfer, membranes can be rinsed in water, dried, and stored between sheets of filter paper at room temperature for months or longer.
3. Optional: Confirm protein transfer by imaging total protein prestain (if used).
4. If using PVDF membranes, re-wet the membrane in methanol, then rinse in water. For nitrocellulose membranes, proceed to step 5.
5. Place blot in a clean dish containing blocking buffer of your choice. Use enough buffer to completely cover the blot and allow it to move freely in the dish.
6. Block membrane for 30 minutes to 1 hour at room temperature with gentle rocking.
7. Dilute antibody conjugate in fresh blocking buffer. Pour off the blocking buffer and add enough diluted antibody solution to allow the membrane to move freely with no stationary bubbles or dry spots.  
**Note:** Antibody conjugate concentration must be optimized for different targets. We recommend 0.5-2 ug/mL as a starting range for optimization for near-IR WB.
8. Incubate membrane with gentle rocking for 1-2 hours at room temperature or overnight at 4°C. Protect from light.
9. Wash membrane 3 times for 5 minutes with rocking in PBS or TBS with 0.1% Tween®-20. Use a generous amount of wash buffer so blots move freely during washes.
10. Rinse blot once in buffer without detergent and dry before imaging using a compatible fluorescence imaging system.  
**Notes:**
  - a. Dried blots can be stored between sheets of filter paper at room temperature in the dark and re-scanned after months or even years.
  - b. Keep blots wet at all times and store in buffer if they are to be stripped and probed with additional antibodies.

## Troubleshooting Tips for Antibody Staining

| Problem                                  | Potential Causes/Diagnosis  | Potential Solutions   |
|--|---|---|
| No staining or low signal                | Antibody conjugate not validated for application  | <ul style="list-style-type: none"> <li>Check that the antibody conjugate is recommended for your application.</li> <li>Validate antibody with positive control cell line or tissue that expresses the target.</li> <li>Check that the species reactivity of the antibody is compatible with your sample.</li> </ul>   |
|  | Target protein not expressed  | Check literature or Human Protein Atlas to confirm the protein is expressed in your sample.   |
|  | Antibody concentration too low  | Perform a titration of antibody concentration to find the optimal concentration. The optimal concentration for primary antibodies can vary widely; concentrations for initial testing usually start around 1 µg/mL or higher.   |
|  | Intracellular target not accessible for surface staining for flow cytometry<br>Diagnosis: Check that the antibody epitope is in an extracellular domain of the target protein. Perform intracellular staining to determine if target is localized inside the cell.  | <ul style="list-style-type: none"> <li>Use an antibody raised against an extracellular domain of the protein, or that is validated for surface staining.</li> <li>Perform intracellular staining for intracellular targets.</li> </ul>  |
|  | Fluorescence photobleaching during microscopy   | Use antifade mounting medium. Some fluorescent dyes are more photostable than others, choose photostable dyes like rhodamine-based CF® Dyes for microscopy applications.  |
|  | Imaging settings not compatible with dyes   | Check that you are using the correct excitation/emission settings for the dyes. Note that far-red conjugates are not visible to the human eye, and must be imaged using a CCD camera or confocal microscope.  |
| High background or non-specific staining | Cell or tissue autofluorescence<br><b>Note:</b> Autofluorescence is a major and nearly universal source of background in tissue sections, and also is present in some primary cells and pigmented cell types.<br>Diagnosis: Include an unstained control to determine the level of autofluorescence in your sample. | <ul style="list-style-type: none"> <li>Cellular autofluorescence is high in blue wavelengths, so avoid using blue fluorescent conjugates for low expressing targets. Autofluorescence may also occur in other channels in certain organisms, we recommend always including an appropriate control. Consult the relevant literature for more information.</li> <li>Use TrueBlack® Lipofuscin Autofluorescence Quencher to quench tissue autofluorescence.</li> <li>Amplify your specific signal over background by using indirect immunofluorescence (primary + secondary antibody) or tyramide signal amplification.</li> </ul>   |
|  | Fluorescence cross-talk between channels<br>Diagnosis: For multi-color experiments, perform controls with each stain alone, and image in all channels to determine whether there is fluorescence cross-talk or bleed-through of dye fluorescence between channels.  | <ul style="list-style-type: none"> <li>Choose dyes that are spectrally well-separated for multi-color imaging. Biotium's <a href="#">Spectra Viewer</a> can be useful for this purpose.</li> <li>Multi-color flow cytometry analysis may require fluorescence compensation. See your cytometer user manual for information.</li> <li>Confocal microscopy imaging settings can be optimized to minimize cross-talk by limiting cross-excitation during scanning, or by changing the emission cut-off for different dyes.</li> <li>To minimize DAPI fluorescence in the green channel, reduce the concentration of DAPI, or optimize confocal imaging settings to prevent cross-talk. Far-red nuclear counterstains for the Cy®5 channel, such as RedDot™2 or NucSpot® 640, can be used to avoid this problem.</li> </ul> |
|  | Blotting membrane autofluorescence<br>Diagnosis: Scan an unused blotting membrane next to your western blot to detect membrane autofluorescence.  | Use low fluorescence PVDF for fluorescent western detection. In our experience, nitrocellulose and low fluorescence PVDF membranes show similar background fluorescence, but PVDF can give higher sensitivity, possibly due to higher protein binding.  |
|  | Suboptimal western blot blocking  | Test different blocking agents to find the optimal conditions, or try a blocking buffer specifically designed for fluorescent westerns, like the TrueBlack® WB Blocking Buffer Kit.   |
|  | Insufficient washing of western blots   | Increasing the number of washes can improve background for western blots. Use a generous volume of wash buffer with rocking so blots move freely during washing.  |
|  | Antibody concentration too high<br>Diagnosis: If both signal and background are high, antibody concentration may be too high.   | Perform a titration of antibody concentration to find the optimal concentration. The optimal concentration for primary antibodies can vary widely. See the application protocols for recommended starting concentrations for titration.   |

## Products for Immunofluorescence

Please visit [www.biotium.com](http://www.biotium.com) to view our full selection of products featuring bright and photostable fluorescent CF® Dyes; primary and secondary antibodies; Mix-n-Stain™ antibody labeling kits; streptavidin, phalloidin, and other bioconjugates; and tyramide signal amplification kits.

| Product   | Cat. No. | Features  |
|---|----------|---|
| 4% Paraformaldehyde in PBS, Ready-to-Use Fixative           | 22023    | <ul style="list-style-type: none"> <li>Ready-to-Use, EM-grade, methanol-free fixation buffer</li> <li>No glass ampoules to break, store in original bottle</li> </ul>   |
| Flow Cytometry Fixation/Permeabilization Kit                | 23006    | <ul style="list-style-type: none"> <li>Ready-to-use fixation/permeabilization buffers for intracellular staining</li> </ul>   |
| TrueBlack® IF Background Suppressor System (Permeabilizing) | 23012    | <ul style="list-style-type: none"> <li>Suppress background from non-specific antibody binding and charged fluorescent dyes</li> <li>More efficient than Image-iT® FX; block &amp; permeabilize in just 10 minutes</li> <li>Complete system for blocking, permeabilizing, and antibody dilution</li> <li>For staining of cells or tissue sections</li> </ul> |
| TrueBlack® WB Blocking Buffer Kit                           | 23013    | <ul style="list-style-type: none"> <li>Blocks non-specific background fluorescence over the entire membrane</li> <li>Works as well or better than LI-COR's Odyssey® Blocking Buffer and at a lower cost</li> <li>Compatible with PVDF and nitrocellulose membranes</li> <li>Can be used with fluorophores spanning the visible and NIR spectra</li> </ul>   |

## Products for Immunofluorescence, Continued

|  |                    |   |
|--|--------------------|---|
| TrueBlack® Lipofuscin Autofluorescence Quencher        | 23007              | <ul style="list-style-type: none"> <li>Eliminates lipofuscin autofluorescence with less background than Sudan Black B</li> <li>Reduces background from other sources like red blood cells and extracellular matrix</li> <li>Can be used before or after IF staining</li> </ul>  |
| TrueBlack® Plus Lipofuscin Autofluorescence Quencher   | 23014              |   |
| EverBrite™ Mounting Medium                             | 23001-23002        | <ul style="list-style-type: none"> <li>Excellent protection from photobleaching for a wide range of dyes, including cyanine (Cy® dyes)</li> <li>Available in wet-set or hardset formulations</li> <li>Drop-n-Stain™ EverBrite packaged in dropper bottles for easy dispensing</li> <li>With or without DAPI</li> </ul>  |
| EverBrite™ Hardset Mounting Medium                     | 23003-23004, 23016 |   |
| Drop-n-Stain EverBrite™ Mounting Medium                | 23008-23009        | <ul style="list-style-type: none"> <li>EverBrite™ Hardset available with far-red NucSpot® 640 nuclear counterstain, avoids cross-talk and UV photoconversion problems with DAPI</li> </ul>  |
| EverBrite TrueBlack® Hardset Mounting Medium           | 23017-23019        | <ul style="list-style-type: none"> <li>The only mounting medium with autofluorescence quenching</li> <li>Quenches as it hardens, with low background</li> </ul>   |
| CoverGrip™ Coverslip Sealant                           | 23005              | <ul style="list-style-type: none"> <li>Superior alternative to nail polish for coverslip sealing</li> <li>Won't mix with aqueous mounting media</li> </ul>  |
| RedDot™2 Far Red Nuclear Counterstain                  | 40061              | <ul style="list-style-type: none"> <li>Far-red nuclear dye for the Cy®5 channel</li> <li>Better nuclear specificity compared to Draq®7</li> </ul>   |
| NucSpot® Nuclear Stains                                | 40083...<br>41038  | <ul style="list-style-type: none"> <li>Green to near-IR fluorescent nuclear counterstains for fixed cells or tissue sections</li> <li>Nuclear-specific, unlike TOTO®, TO-PRO®, or SYTOX® dyes</li> </ul>  |
| Live-or-Dye™ Fixable Viability Stains                  | 32002...<br>32018  | <ul style="list-style-type: none"> <li>Fixable dead cell stains compatible with downstream immunofluorescence staining</li> <li>Exclude dead cells from flow cytometry analysis</li> <li>Suitable for microscopy</li> </ul>   |
| Live-or-Dye NucFix™ Red                                | 32010              | <ul style="list-style-type: none"> <li>Fixable nuclear dead cell stain</li> <li>Exclude dead cells from flow cytometry analysis</li> <li>Suitable for microscopy</li> </ul>   |
| VersaBlot™ Total Protein Normalization Kit             | 33025,<br>33026    | <ul style="list-style-type: none"> <li>Superior linearity for western normalization compared to housekeeping proteins</li> <li>Reversible prestain for downstream multi-color western blot analysis</li> <li>Highly sensitive protein quantitation on PAGE gels (≤ 1 ng) or western membranes</li> <li>Easily label purified proteins or cell lysates before SDS-PAGE</li> <li>Near-infrared fluorescence for Typhoon™ or Odyssey®</li> </ul> |
| Peacock™ Prestained Protein Marker                     | 21530              | <ul style="list-style-type: none"> <li>Protein ladders for SDS-PAGE with blue and red visible bands ranging from 8 kDa to 245 kDa</li> <li>Ladders also fluoresce in the far-red, convenient for fluorescent western blotting</li> </ul>  |
| Peacock™ Plus Prestained Protein Marker                | 21531              |   |
| Mini-Cell Scrapers                                     | 22003              | <ul style="list-style-type: none"> <li>Collect cells from 96-well to 24-well plates</li> <li>Polypropylene, sterile, disposable</li> </ul>  |
| 4X Protein Loading Buffer with Orange Tracking Dye     | 40136              | <ul style="list-style-type: none"> <li>Convenient buffers, blocking agents, and accessories for immunofluorescence, flow cytometry, or western blotting</li> </ul>  |
| Fixation Buffer  | 22015              |   |
| Flow Cytometry Fixation/Permeabilization Kit           | 23006              |   |
| Permeabilization Buffer                                | 22016              |   |
| Permeabilization and Blocking Buffer                   | 22017              |   |
| 10X Fish Gelatin Blocking Agent                        | 22010              |   |
| Fish Gelatin Powder                                    | 22011              |   |
| 30% Bovine Serum Albumin Solution                      | 22014              |   |
| Tween®-20  | 22002              |   |
| Ponceau S Solution                                     | 22001              |   |
| Mini Super <sup>HT</sup> Pap Pen 2.5 mm tip, ~400 uses | 22005              |   |
| Super <sup>HT</sup> Pap Pen 4 mm tip, ~800 uses        | 22006              |   |

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