

Produktinformation



Forschungsprodukte & Biochemikalien
Zellkultur & Verbrauchsmaterial
Diagnostik & molekulare Diagnostik
Laborgeräte & Service

Weitere Information auf den folgenden Seiten! See the following pages for more information!



Lieferung & Zahlungsart siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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www.biotium.com





Product Information

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

Ex/Em (nm)	Cat. No.	Size
. ,	20844-50uL	50 uL
411/431 nm	20844-500uL	500 uL
	20844-1mg	1 mg (lyophilized)
490/516 nm	20845-50uL	50 uL
	20845-500uL	500 uL
	20845-1mg	1 mg (lyophilized)
543/563 nm	20846-50uL	50 uL
	20846-500uL	500 uL
	20846-1mg	1 mg (lyophilized)
554/568 nm	20847-50uL	50 uL
	20847-500uL	500 uL
	20847-1mg	1 mg (lyophilized)
	20848-50uL	50 uL
562/584 nm	20848-500uL	500 uL
	20848-1mg	1 mg (lyophilized)
	20849-50uL	50 uL
593/615 nm	20849-500uL	500 uL
	20849-1mg	1 mg (lyophilized)
	20850-50uL	50 uL
642/663 nm	20850-500uL	500 uL
	20850-1mg	1 mg (lyophilized)
	20851-50uL	50 uL
652/668 nm	20851-500uL	500 uL
	20851-1mg	1 mg (lyophilized)
	20852-50uL	50 uL
667/685 nm	20852-500uL	500 uL
	20852-1mg	1 mg (lyophilized)
	20853-50uL	50 uL
662/682 nm	20853-500uL	500 uL
	20853-1mg	1 mg (lyophilized)
	20854-50uL	50 uL
681/698 nm	20854-500uL	500 uL
	20854-1mg	1 mg (lyophilized)
680/701 nm	20855-50uL	50 uL
	20855-500uL	500 uL
	20855-1mg	1 mg (lyophilized)
	20856-50uL	50 uL
755/779 nm	20856-500uL	500 uL
	20856-1mg	1 mg (lyophilized)
N/A	20475-100uL	100 uL
	20475-1mL	1 mL
	20475-1mg	1 mg (lyophilized)
	20983-50uL	50 uL
N/A	20983-500uL	500 uL
	411/431 nm 490/516 nm 543/563 nm 554/568 nm 562/584 nm 642/663 nm 652/668 nm 667/685 nm 667/685 nm 662/682 nm 681/698 nm 681/698 nm 755/779 nm N/A	20844-50uL 411/431 nm 20844-50uL 20844-1mg 20845-50uL 490/516 nm 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20845-50uL 20846-1mg 20847-50uL 20847-50uL 554/568 nm 20847-50uL 562/584 nm 20848-50uL 20848-50uL 20848-50uL 562/584 nm 20849-50uL 20849-500uL 20849-500uL 20849-500uL 20849-500uL 20850-50uL 20850-50uL 662/663 nm 20850-500uL 665/665 nm 20851-500uL 667/685 nm 20852-500uL 662/682 nm 20852-500uL 662/682 nm 20853-500uL 662/682 nm 20853-500uL 681/698 nm 20853-500uL 681/698 nm 20855-500uL 680/701 nm 20855-500uL 680/701 nm 20856-500uL 20855-500uL

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 dH₂O 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 dH₂O 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:

- a. Do not add sodium azide to HRP conjugates.
- b. 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-Ilama 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 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.

Recommended Dilution Range

- Fluorescence microscopy: 1-2 ug/mL
- Flow cytometry: 1 ug/10⁶ 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 <u>Tech Tip: Considerations for Immunofluorescence Staining</u> 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 Ca²⁺/Mg²⁺ 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 (Ca²⁺/Mg²⁺-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

- 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 Ca²⁺/Mg²⁺ may be necessary to prevent cell rounding and detachment. Prior to fixation, we prefer to use HBSS + Ca²⁺/Mg²⁺ for adherent cells.
- 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).

 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.

 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.

 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.

 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.

 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.
- 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 lodide (also known as TO-PRO®-1), can be added together with primary antibody. <u>View our full selection of non-fixable dead cell stains</u>.

- 3. Adjust cell density to 10⁷ cells per mL in flow buffer.
- 4. Aliquot 100 uL of cell suspension per flow cytometry tube for a total of 10^6 cells per tube. Place tubes on ice.
- 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.

- 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).
- 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

- Detach adherent cells from substrate by trypsinization or with a commercial non-enzymatic cell lift solution.
- 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.
- Optional: Perform antibody staining for cell surface markers (see Cell Surface Antibody Staining for Flow Cytometry).
- 4. Adjust cell density to 107 cells per mL in PBS.
- Aliquot 100 uL cell suspension to each 12 x 75 mm polypropylene flow cytometry tubes for a total of 10⁶ cells per tube.
- 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 cm² 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

- 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.
- 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.
- Block membrane for 30 minutes to 1 hour at room temperature with gentle rocking.
- 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.

- Incubate membrane with gentle rocking for 1-2 hours at room temperature or overnight at 4°C. Protect from light.
- 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:

- 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	
	Antibody conjugate not validated for application	 Check that the antibody conjugate is recommended for your application. Validate antibody with positive control cell line or tissue that expresses the target. Check that the species reactivity of the antibody is compatible with your sample. 	
	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 ug/mL or higher.	
No staining	Intracellular target not accessible for surface staining for flow cytometry	 Use an antibody raised against an extracellular domain of the protein, or that is validated for surface staining. Perform intracellular staining for intracellular targets. 	
or low signal	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.		
	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.	
	Cell or tissue autofluorescence	Cellular autofluorescence is high in blue wavelengths, so avoid using blue fluorescent	
High background or non-specific staining	Note: 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.	 Central automotescence is high in blue wavelengths, so avoid using blue inderescent 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. Use TrueBlack® Lipofuscin Autofluorescence Quencher to quench tissue autofluorescence. Amplify your specific signal over background by using indirect immunofluorescence 	
	Diagnosis: Include an unstained control to determine the level of autofluorescence in your sample.	(primary + secondary antibody) or tyramide signal amplification.	
	Fluorescence cross-talk between channels 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.	 Choose dyes that are spectrally well-separated for multi-color imaging. Biotium's <u>Spectra Viewer</u> can be useful for this purpose. Multi-color flow cytometry analysis may require fluorescence compensation. See your cytometer user manual for information. 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. 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. 	
	Blotting membrane autofluorescence	Use low fluorescence PVDF for fluorescent western detection. In our experience, nitrocellulose	
	Diagnosis: Scan an unused blotting membrane next to your western blot to detect membrane autofluorescence.	and low fluorescence PVDF for fluorescent western detection. In our experience, hitrocellulose 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 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 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	 Ready-to-Use, EM-grade, methanol-free fixation buffer No glass ampoules to break, store in original bottle
Flow Cytometry Fixation/Permeabilization Kit	23006	Ready-to-use fixation/permeabilization buffers for intracellular staining
TrueBlack® IF Background Suppressor System (Permeabilizing)	23012	 Suppress background from non-specific antibody binding and charged fluorescent dyes More efficient than Image-iT® FX; block & permeabilize in just 10 minutes Complete system for blocking, permeabilizing, and antibody dilution For staining of cells or tissue sections
TrueBlack® WB Blocking Buffer Kit	23013	 Blocks non-specific background fluorescence over the entire membrane Works as well or better than LI-COR's Odyssey® Blocking Buffer and at a lower cost Compatible with PVDF and nitrocellulose membranes Can be used with fluorophores spanning the visible and NIR spectra

Products for Immunofluorescence, Continued

Products for Immunofluorescence, Continue	d		
TrueBlack® Lipofuscin Autofluorescence Quencher	23007	Eliminates lipofuscin autofluorescence with less background than Sudan Black B Reduces background from other sources like red blood cells and extracellular matrix Can be used before or after IF staining	
TrueBlack® Plus Lipofuscin Autofluorescence Quencher	23014		
EverBrite™ Mounting Medium	23001-23002	 Excellent protection from photobleaching for a wide range of dyes, including cyanine (Cy® dyes) Available in wet-set or hardset formulations Drop-n-Stain™ EverBrite packaged in dropper bottles for easy dispensing With or without DAPI EverBrite™ Hardset qualitable with for red NusSect® 640 pueleer countertain qualitable 	
EverBrite™ Hardset Mounting Medium	23003- 23004, 23016		
Drop-n-Stain EverBrite™ Mounting Medium	23008-23009		
EverBrite TrueBlack® Hardset Mounting Medium	23017-23019	 The only mounting medium with autofluorescence quenching Quenches as it hardens, with low background 	
CoverGrip™ Coverslip Sealant	23005	Superior alternative to nail polish for coverslip sealingWon't mix with aqueous mounting media	
RedDot™2 Far Red Nuclear Counterstain	40061	 Far-red nuclear dye for the Cy®5 channel Better nuclear specificity compared to Draq®7 	
NucSpot® Nuclear Stains	40083 41038	 Green to near-IR fluorescent nuclear counterstains for fixed cells or tissue sections Nuclear-specific, unlike TOTO®, TO-PRO®, or SYTOX® dyes 	
Live-or-Dye™ Fixable Viability Stains	32002 32018	 Fixable dead cell stains compatible with downstream immunofluorescence staining Exclude dead cells from flow cytometry analysis Suitable for microscopy 	
Live-or-Dye NucFix™ Red	32010	 Fixable nuclear dead cell stain Exclude dead cells from flow cytometry analysis Suitable for microscopy 	
VersaBlot™ Total Protein Normalization Kit	33025, 33026	 Superior linearity for western normalization compared to housekeeping proteins Reversible prestain for downstream multi-color western blot analysis Highly sensitive protein quantitation on PAGE gels (≤ 1 ng) or western membranes Easily label purified proteins or cell lysates before SDS-PAGE Near-infrared fluorescence for Typhoon™ or Odyssey® 	
Peacock™ Prestained Protein Marker	21530		
Peacock™ Plus Prestained Protein Marker	21531	 Protein ladders for SDS-PAGE with blue and red visible bands ranging from 8 kDa to 245 kDa Ladders also fluoresce in the far-red, convenient for fluorescent western blotting 	
Mini-Cell Scrapers	22003	Collect cells from 96-well to 24-well plates Polypropylene, sterile, disposable	
4X Protein Loading Buffer with Orange Tracking Dye	40136		
Fixation Buffer	22015		
Flow Cytometry Fixation/Permeabilization Kit	23006	1	
Permeabilization Buffer	22016		
Permeabilization and Blocking Buffer	22017	Convenient buffers, blocking agents, and accessories for immunofluorescence, flow cytometry, or western blotting	
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 ^{HT} Pap Pen 2.5 mm tip, ~400 uses	22005		
Super ^{н⊤} Pap Pen 4 mm tip, ~800 uses	22006		

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