Product Information

Basic Protocols for Antibody-Based Detection

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Note: 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

- This is a protocol for staining of 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®350, CF®405S, and CF®405M, are 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^{2+}/Mg^{2+} may be optimal for adherent cells)
- Paraformaldehyde, 4% in PBS (Cat. No. 22023), or methanol pre-chilled to -20°C (see notes to step 2 of Procedure)
- 1X PBS (Ca^{2+}/Mg^{2+}-free is acceptable)
- PBS + 2% fish gelatin + 0.1% Triton® X-100 + 0.02% sodium azide
- Primary antibody
- Secondary antibody (not necessary if using labeled primary antibody)
- Antifade mounting medium
- Coverslip sealant (for wet-mounted coverslips only)

Workflow overview

- Fix (≤ 20 minutes) *(optional stopping point)*
- Block/permeabilize (30 minutes) *(optional stopping point)*
- Primary antibody incubation (1-2 hours or overnight)
- Washes (20-30 minutes)
- Secondary antibody incubation (30 minutes to 2 hours) (not necessary if using labeled primary antibody)
- 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^{2+}/Mg^{2+} may be necessary to prevent cell rounding and detachment. Prior to fixation, we prefer to use HBSS + Ca^{2+}/Mg^{2+} for adherent cells.
2. Fix cells with 4% paraformaldehyde in PBS for 20 minutes at room temperature. Alternatively, fix cells in pre-chilled methanol at -20°C for 5-10 minutes.

   Notes:

   a. Check the information provided by the primary antibody supplier to see if a specific fixation method is recommended. If the optimal fixation conditions are unknown, it may be necessary to test different fixation methods for a specific antibody or target epitope.

   b. Methanol fixation is not compatible with phalloidin staining.
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.

   Optional: You can store samples at 4°C for several weeks at this point. Keep samples well-sealed or in a humidified box to avoid evaporation of buffer.

   Note: Other blocking reagents such as BSA may also be used instead of fish gelatin. When using some highly negative charged fluorescent dyes, specialized blocking buffers, such as our TrueBlack® IF Background Suppressor System (Cat. No. 23012), may help reduce background.

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5. Dilute primary antibody in fresh blocking/permeabilization buffer at the concentration recommended by the antibody supplier.

   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). If using fluorescently labeled primary antibodies, protect samples from light.

   Notes:

   a. Due to their fast binding on-rates, MiniMab™ VHH nanobodies may require shorter incubation times relative to IgG antibodies. They may only require a 30 minute incubation instead of 1 hour to overnight.

   b. Other stains, such as nuclear counterstains, lectins, or phalloidin conjugates, can be added together with labeled antibodies at this step, or at step 10 if using labeled secondary antibodies.

8. Rinse cells twice with PBS, then wash 3 times for 5 minutes each 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 for longer times without negatively affecting staining.

9. If using directly labeled primary antibodies, proceed to step 12. If using labeled secondary antibodies, proceed to step 10.

10. Dilute secondary antibody in blocking/permeabilization buffer at 1 ug/mL. Cover cells with secondary antibody solution as in step 5 and incubate for 30 minutes to 2 hours at room temperature, protected from light.

11. Wash cells as in step 8.

12. 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, wetset 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 (Cat. No. 23005) (recommended) before imaging.

13. Store samples at 4°C, protected from light, until ready to image. Samples can be stored in mounting medium at 4°C for six months or longer.

    Note: Phalloidin staining is less stable than antibody staining. Staining with most phalloidin conjugates is stable at 4°C for several days, but for best results it should be imaged within 24 hours.

Cell Surface Staining for Flow Cytometry

Materials required

- Live-or-Dye™ Fixable Viability Stain or dead cell nucleic acid stain (optional)
- Primary antibody
- Secondary antibody (not necessary if using labeled primary antibody)
- 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
- Optional: Dead cell staining
- Primary antibody incubation (30 minutes)
- Wash and centrifuge (2 x 5 minutes)
- Optional: Fixation followed by wash
- Secondary antibody incubation (30 minutes) (not necessary if using labeled primary antibody)
- Wash and centrifuge (2 x 5 minutes) *(optional stopping point if fixation is used)*
- 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 or secondary antibody. View our full selection of non-fixable dead cell stains.

3. Adjust cell density to 10^7 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.

5. Add primary antibodies to tubes and vortex gently to mix. Incubate tubes on ice (or at 4°C) for 30 minutes. If using directly conjugated fluorescent primary antibodies, tubes should be protected from light.

   Note: Primary antibody concentration must be optimized for different applications, 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.

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7. Pour off the supernatant 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. If using labeled primary antibodies, proceed to step 14. If using labeled secondary antibodies, continue with step 11.
11. After pouring off supernatant, resuspend cells in residual buffer (~100 uL) by gentle vortexing.
12. Add 1 ug of each secondary antibody to each tube and vortex gently to mix. Incubate at room temperature, protected from light, for 30 minutes.

    Note: For biotinylated primary antibodies, Streptavidin Conjugates can be used for detection, typically at 0.25 ug/tube.
13. Wash cells twice in flow buffer (repeat steps 6-7).
14. After pouring off supernatant, add 500 uL flow buffer per tube.
15. 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 (optional)
- Flow Cytometry Fixation/Permeabilization Kit (Cat. No. 23006) (optional)
- Primary antibody
- Secondary antibody (not necessary if using labeled primary antibody)
- 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
- Optional: Dead cell staining
- Fixation (20 minutes)
- Wash and centrifuge (5 minutes)
- Permeabilization/primary antibody incubation (30 minutes)
- Wash and centrifuge (2 x 5 minutes)
- Secondary antibody incubation (30 minutes) (not necessary if using labeled primary antibody)
- Wash and centrifuge (2 x 5 minutes) *(optional stopping point)*
- 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 10^7 cells per mL in PBS.
5. Aliquot 100 uL cell suspension to each flow cytometry tube for a total of 10^6 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. Pour off the supernatant into a waste container.
9. Add 100 uL permeabilization buffer to each tube and mix by gentle vortexing.
10. Add primary antibodies to the tubes and vortex gently to mix. Incubate at room temperature for 30 minutes. If using fluorescent labeled primary antibodies, protect samples from light.

    Note: Primary antibody concentration must be optimized for different applications, 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. Pour off the supernatant into a waste container and repeat step 11.
13. If using labeled primary antibodies, proceed to step 17. If using secondary antibodies, continue with step 14.
14. After pouring off supernatant, resuspend cells in the residual buffer (~100 uL) by gentle vortexing.
15. Add 1 ug secondary antibodies to each tube and vortex gently to mix. Incubate at room temperature, protected from light, for 30 minutes.

    Note: For biotinylated primary antibodies, Streptavidin Conjugates can be used for detection, typically at 0.25 ug/tube.
16. Wash cells twice in flow buffer (repeat step 11-12).
17. After pouring off supernatant, add 500 uL of flow buffer per tube.
18. 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.

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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% sodium azide (see general considerations below)
- Primary antibody
- Secondary antibody (not necessary if using labeled primary antibody)

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)
- Primary antibody incubation (2 hours or overnight)
- Washes (~15-30 minutes)
- Secondary antibody incubation (30 minutes to 2 hours) (not necessary if using labeled primary antibody)
- Washes (~15-30 minutes)
- Dry membrane *(optional stopping point)*
- Image membrane

General considerations for fluorescent western detection

- 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.
- Check the primary antibody supplier's recommendations and literature to determine whether you should use reducing or non-reducing SDS-PAGE for your target/antibody of interest.
- 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 (Cat. No. 21530, 21531), which have bands that range from 8 kDa to 245 kDa. The bands fluoresce in the 700 channel and are visibly 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.
- 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 (Cat. No. 40136) for fluorescent western blots.
- 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 (Cat. No. 33025, 33026) due to their exceptional linearity, ease-of-use, and downstream reversibility for multi-color analysis.
- 9 cm^2 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 (Cat. No. 23013A-500mL), 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.
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.

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7. Dilute primary antibody to recommended concentration 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.
8. Incubate membrane with gentle rocking for 1-2 hours at room temperature or overnight at 4°C. If using fluorescently labeled primary antibodies, protect from light.
9. Wash membrane 3 times for 5 minutes each 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. If using fluorescently labeled primary antibodies, continue to step 13. If using labeled secondary antibody conjugates, continue to step 11.
11. Dilute secondary antibody in fresh blocking buffer at the concentration recommended by the supplier for western blot (usually in the range of 50-100 ng/mL). Add to blot as in step 7. Incubate 30 minutes to 2 hours with rocking.

    Note: Some near-IR secondary antibody conjugates require additional detergent to be added to the buffer, check the supplier instructions for your antibody conjugate and blocking buffer for recommendations.
12. Wash membrane as in step 9.
13. 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	Primary antibody not validated for application	• Check that primary antibody 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. Secondary antibodies are typically used at 1 ug/mL for cell staining and as low as 50 ng/mL for near-infrared western detection.
	Intracellular target not accessible for surface staining for flow cytometry 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.	• Use an antibody raised against an extracellular domain of the protein, or that is validated for surface staining. • Perform intracellular staining for intracellular targets.
	Wrong type of fixation protocol used	Check the protocol supplied with the primary antibody and refer to the literature to see if solvent or PFA is used for fixation, or perform a side-by-side comparison to determine the optimal fixation method for your experiment.
	Secondary antibodies not compatible with serum proteins used for blocking	If using anti-goat or anti-bovine secondary antibodies, avoid blocking buffers with milk, goat serum, or bovine serum albumin.
	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.

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Troubleshooting Tips for Antibody Staining (continued)

Problem	Potential Causes/Diagnosis	Potential Solutions
High background or non-specific staining	Cell or tissue autofluorescence **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. Include an unstained control to determine the level of autofluorescence in your sample.	• Cellular autofluorescence is high in blue wavelengths, so avoid using blue fluorescent conjugates for low expressing targets. Autofluorescence may also occur in other wavelengths in certain organisms. We recommend always including an appropriate control or consult the relevant literature. • Use TrueBlack® Lipofuscin Autofluorescence Quencher (Cat. No. 23007, 23011) to quench tissue autofluorescence. • Amplify your specific signal over background by using indirect immunofluorescence (primary + secondary antibody) or tyramide signal amplification.
	Cross-reactivity of secondary antibody with other antibodies or proteins in sample Perform staining controls with secondary antibody alone to determine whether the secondary antibody is binding the sample directly. For multiple staining experiments, stain with each primary and secondary combination separately to detect unexpected antibody cross-reactivity.	• For indirect staining (primary + secondary antibody) with multiple primary antibodies, use secondary antibodies that are highly cross-adsorbed to prevent cross-reactivity. • When staining rat tissue with anti-mouse antibodies, use secondary antibodies that are highly cross-adsorbed against rat. • Staining of mouse tissues with anti-mouse antibodies (known as mouse-on-mouse staining) may require special protocols to block binding of endogenous antibodies in the tissue. • Highly charged fluorescent dyes, including CF®405S, Alexa Fluor® 647, and Cy®5.5 can contribute to non-specific binding of conjugates. Specialized blocking buffers, such as TrueBlack® IF Background Suppressor System (Cat. No. 23012) or TrueBlack® WB Blocking Buffer (Cat. No. 23013A-500ML), can reduce background from charged dyes.
	Fluorescence cross-talk between channels 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 multicolor imaging. Biotium's Spectra Viewer 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. Nuclear counterstains for other channels, such as RedDot™2 or NucSpot® Nuclear Stains, can be used to avoid this problem.
	Blotting membrane autofluorescence 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 (Cat. No. 23013).
	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 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; concentrations for initial testing usually start around 1 ug/mL or higher (up to 5 ug/mL for direct conjugates). Secondary antibodies are typically used at 1 ug/mL for cell staining and as low as 50 ng/mL for near-infrared western detection.

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Products for Immunofluorescence

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
TrueBlack® Lipofuscin Autofluorescence Quencher	23007, 23011	• 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 available with far-red NucSpot® 640 nuclear counterstain, avoids cross-talk and UV photoconversion problems with DAPI
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 sealing • Won'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 fluorescent nuclear counterstain 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	• 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
Peacock™ Plus Prestained Protein Marker	21531	

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Products for Immunofluorescence (continued)

Product	Cat. No.	Features
4X Protein Loading Buffer with Orange Tracking Dye	40136	• Convenient buffers, blocking agents, and accessories for immunofluorescence or western
Fixation Buffer	22015	
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	
Super^{HT} Pap Pen 2.0	23023, 23024	

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; bioconjugates; and tyramide signal amplification kits.

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