Product Information

ExoBrite™ Western Antibody Conjugates

Product List

Cat. No.	Product	Unit Size
P003-680-250	ExoBrite™ 680/700 CD9 Western Antibody	25 tests
P003-680-1000	ExoBrite™ 680/700 CD9 Western Antibody	100 tests
P003-770-250	ExoBrite™ 770/800 CD9 Western Antibody	25 tests
P003-770-1000	ExoBrite™ 770/800 CD9 Western Antibody	100 tests
P003-HRP-500UL	ExoBrite™ HRP CD9 Western Antibody	50 tests
P004-680-250	ExoBrite™ 680/700 CD63 Western Antibody	25 tests
P004-680-1000	ExoBrite™ 680/700 CD63 Western Antibody	100 tests
P004-770-250	ExoBrite™ 770/800 CD63 Western Antibody	25 tests
P004-770-1000	ExoBrite™ 770/800 CD63 Western Antibody	100 tests
P004-HRP-500UL	ExoBrite™ HRP CD63 Western Antibody	50 tests
P006-680-250	ExoBrite™ 680/700 CD81 Western Antibody	25 tests
P006-680-1000	ExoBrite™ 680/700 CD81 Western Antibody	100 tests
P006-770-250	ExoBrite™ 770/800 CD81 Western Antibody	25 tests
P006-770-1000	ExoBrite™ 770/800 CD81 Western Antibody	100 tests
P005-HRP-500UL	ExoBrite™ HRP CD81 Western Antibody	50 tests
P007-770-250	ExoBrite™ 770/800 Calnexin Western Antibody	25 tests
P007-770-1000	ExoBrite™ 770/800 Calnexin Western Antibody	100 tests

Note: Fluorescent conjugates are supplied in PBS, 0.1% BSA, and 0.05% azide. HRP conjugates are supplied in PBS, 50% glycerol, and 2 mg/mL rBSA.

Storage and Handling

Store fluorescent conjugates at 4°C, protected from light. Store HRP conjugates at -20°C, protected from light. Product is stable for at least 24 months from date of receipt when stored as recommended.

Note: Storage of the antibody for more than a day at final working dilution is not recommended.

Product Description

The most common proteins used as extracellular vesicle (EV) markers are CD9, CD63, and CD81, members of the tetraspanin family. Tetraspanins are plasma membrane proteins with many proposed functions, including activation and sorting of other membrane proteins. They are also thought to play a role in the targeting of proteins to multivesicular bodies (MVBs) and EVs. These tetraspanins are broadly expressed on many cell types and can therefore be detected on many types of EVs, but their expression levels vary depending on the cell type of origin.

ExoBrite™ Western Antibody Conjugates are validated by Biotium for optimal detection of EV markers CD9, CD63, and CD81 in isolated EV extracts by fluorescent or chemiluminescent western blot. The antibodies offer exceptional performance, providing greater sensitivity than indirect detection using an unlabeled primary antibody and a labeled secondary antibody. The antibodies are conjugated to near-infrared (near-IR) fluorescent CF® Dyes or HRP, which offer greater signal-to-noise than dyes with visible light emission for western blotting.

ExoBrite™ Calnexin Western Antibody detects a protein of the endoplasmic reticulum that is not found in EVs. It is offered as a negative control to assess the purity of isolated EV extracts.

Biotium offers a wide variety of ExoBrite™ products for the study of EVs. See Related Products or our technology page.

General Considerations for EV Isolation for Western Blotting

- Obtaining a clean EV prep is crucial for obtaining robust signal and proper interpretation of results. While there are several EV isolation methods, we have found that size exclusion chromatography (SEC) is an accessible and easy-to-use method that yields a relatively pure population of EVs. For detailed protocols on EV isolation by SEC or PEG precipitation, read our Tech Tip: Isolation and Staining of Extracellular Vesicles. For a detailed comparison of EV isolation methods, read our Tech Tip: Fluorescent Detection of EVs by Flow Cytometry.

Table 1. Antibody Attributes

Antibody	Target	Host species	Species reactivity	Target MW	Target localization	Isotype	Entrez gene ID	SwissProt	Unigene	Synonyms
ExoBrite™ CD9 Western Antibody	CD9	Mouse	Human, Baboon, Bovine, Cynomolgus monkey, Dog, Horse, Rabbit, Non-human primates, Sheep	24 kDa	Exosomes/EVs, Plasma membrane	IgG1, kappa	928	P21926	114286	Tspan-29, MRP-1
ExoBrite™ CD63 Western Antibody	CD63	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates	26 kDa (core protein); 30-60 kDa (glycosylated)	Exosomes/EVs, Lysosomes, Plasma membrane, Membrane/vesicular, Multivesicular bodies	IgG1, kappa	967	P08962	445570	Tspan-30, LAMP-3, gp55
ExoBrite™ CD81 Western Antibody	CD81	Mouse	Human, Baboon, Cynomolgus monkey, Non-human primates	26 kDa	Exosomes/EVs, Plasma membrane	IgG1, kappa	975	P60033	54457	Tspan-28, TAPA-1
ExoBrite™ Calnexin Western Antibody	Calnexin	Mouse	Human	67 kDa (predicted); 80-90 kDa (observed)	Endoplasmic reticulum (negative control for EV purity assessment)	IgG1, kappa	821	P27824	567968	IP90

- Optimal protein loading amount will depend on the expression level of the target, which varies between cell type and sample type. As a starting point for optimization, we recommend loading 1-10 ug per lane of total protein from cell lysates. Optimal loading for EV lysates may be lower than for cell lysates, because tetraspanins are enriched in EVs. We recommend testing 1-3 ug of protein from purified EVs as a starting point for optimization. Higher EV protein amounts may be required depending on the target expression level in your sample.

General Considerations for Fluorescent Western Blotting

- Multiplex fluorescence western detection requires an imaging system capable of detecting fluorescent dyes in multiple channels. Use a gel imager or scanner specifically designed for imaging fluorescent blots. See Table 2 for detection settings for common fluorescent gel imaging systems.
- Far-red or near-IR dyes, such as ExoBrite™ 680/700 and ExoBrite™ 770/800, 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.
- As a protein marker, we recommend using Peacock™ Prestained Protein Markers which fluoresce in the 700 channel and range from 8 kDa to 245 kDa (Cat. No. 21530, 21531). We recommend loading 1.5-3 uL per lane of ladder for optimal visualization alongside ExoBrite™ Western Antibodies.
- Blue tracking dyes in SDS-PAGE loading buffer can fluoresce in the far-red/near-IR spectra; loading buffer with an orange tracking dye is recommended for fluorescent western detection, such as 4X Protein Loading Buffer with Orange Tracking Dye (Cat. No. 40136).
- 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 multicolor analysis (see Related Products).
- 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.
- For blocking, we recommend using TrueBlack® WB Blocking Buffer Kit, which was developed specifically for fluorescent western detection and has shown the best performance with ExoBrite™ 680/700 and ExoBrite™ 770/800. BSA, non-fat dry milk, and fish gelatin may also be used for western blot blocking and antibody dilution buffers (see Related Products). These blocking agents are usually used at 1-5% in PBS (or TBS) + 0.1% Tween® 20.
- 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, don't skimp on volume. Use 5-10 mL buffer for a mini-blot. The blot should move freely in the buffer.

General Considerations for Chemiluminescent Western Blotting

- HRP function is inhibited by azides, so chemiluminescent western blotting is not compatible with azide in blocking buffers and our TrueBlack® WB Blocking Buffer is not suitable. BSA, non-fat dry milk, and fish gelatin may also be used for western blot blocking and antibody dilution buffers (see Related Products). These blocking agents are usually used at 1-5% in PBS (or TBS) + 0.1% Tween® 20.

- The sensitivity of the western blot and the concentration of antibody needed will vary depending on the sensitivity of the enhanced chemiluminescence (ECL) substrate used. We recommend 100 ng/mL as a starting point for titration with a femtogram-sensitive substrate.
- Chemiluminescent detection must be done using x-ray film or a blot imaging system that can image chemiluminescence.
- 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 chemiluminescent western detection with similar results.
- 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, don't skimp on volume. Use 5-10 mL buffer for a mini-blot. The blot should move freely in the buffer.

Fluorescent Western Blotting Protocol

Materials required but not provided

- Optional: VersaBlot™ Total Protein Normalization Kit (Cat. No. 33025, 33026)
- TrueBlack® WB Blocking Buffer Kit (Cat. No. 23013) or other blocking buffer
- PBS or TBS with 0.1% Tween® 20
- Peacock™ Prestained Protein Markers (Cat. No. 21530, 21531) or other protein ladder

Workflow overview

1. Optional: Perform total protein prestaining
2. Perform SDS-PAGE and transfer to membrane (~2 hours) *(optional stopping point)*
3. Optional: Confirm protein transfer
4. Blocking (30-60 minutes)
5. Primary antibody incubation (2 hours or overnight)
6. Washes (30 minutes)
7. Optional: Secondary antibody incubation (not required for labeled primary antibody) (30 minutes to 2 hours)
8. Optional: Washes (~30-60 minutes) (required only if using secondary antibodies)
9. Dry membrane *(optional stopping point)*
10. Image membrane

Detailed protocol

1. Optional: To fluorescently label total protein for transfer confirmation and normalization, use a total protein prestain kit, such as our VersaBlot™ Total Protein Normalization Kit (Cat. No. 33025, 33026), according to the protocol.
2. Perform SDS-PAGE and western transfer using standard protocols.

   Notes:

   a. Reducing agents must not be used in the SDS-PAGE loading buffer, because the tetraspanin antibodies will not bind the proteins when reduced. Calnexin detection is not affected by reducing agents.

   b. As a protein marker, we recommend using 1.5-3 uL per lane of Peacock™ Prestained Protein Markers (Cat. No. 21530, 21531).

   c. Refer to the General Considerations for Fluorescent Western Detection for considerations on selecting a blotting membrane.

   d. 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: If using VersaBlot™ Total Protein Prestain, image the prestaining signal at this step. If you will be detecting other targets in the same channel used for total protein staining, perform the reversal steps as described in the VersaBlot™ Product Information Sheet.
4. If using PVDF membranes, re-wet the membrane in methanol, then rinse in water. For nitrocellulose membranes, proceed to step 5.
5. Place membrane 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. For optimal blocking, we recommend the TrueBlack® WB Blocking Buffer Kit (Cat. No. 23013).
6. Block membrane for 30-60 minutes at room temperature with gentle rocking.
7. Dilute ExoBrite™ 680/700 or ExoBrite™ 770/800 1:1000 in fresh blocking buffer (if you are using the TrueBlack® WB Blocking Buffer Kit, use the Antibody Diluent at this step). If using other primary antibodies, perform a titration to find the optimal concentration. 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, protected 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 only using ExoBrite™ fluorescently labeled primary antibodies, continue to step 13. If using labeled secondary antibody conjugates with unlabeled primary antibodies, 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 membrane for 30 minutes to 2 hours with rocking.

    Note: Avoid using anti-mouse secondary antibodies because they will also bind ExoBrite™ Western Antibodies.

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 (see Table 2). See Figure 1 for example blots of ExoBrite™ Western Antibody Conjugates in MCF-7 total cell lysates and EV preps.

    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.

Chemiluminescent Western Blotting Protocol

Materials required but not provided

- Blocking buffer
- PBS or TBS with 0.1% Tween® 20
- Protein ladder
- ECL substrate

Workflow overview

1. Perform SDS-PAGE and transfer to membrane (~2 hours) *(optional stopping point)*
2. Optional: Confirm protein transfer
3. Blocking (30-60 minutes)
4. Primary antibody incubation (2 hours or overnight)
5. Washes (30 minutes)
6. Optional: Secondary antibody incubation (not required for labeled primary antibody) (30 minutes to 2 hours)
7. Optional: Washes (~30-60 minutes) (required only if using secondary antibodies)
8. ECL substrate incubation (1-5 minutes)
9. Image membrane

Detailed protocol

1. Perform SDS-PAGE and western transfer using standard protocols.

    Notes:

    a. Reducing agents must not be used in the SDS-PAGE loading buffer, because the tetraspanin antibodies will not bind the proteins when reduced.

    b. After transfer, membranes can be rinsed in water, dried, and stored between sheets of filter paper at room temperature for months or longer.

2. If using PVDF membranes, re-wet the membrane in methanol, then rinse in water. For nitrocellulose membranes, proceed to step 3.
3. Place membrane in a clean dish containing azide-free blocking buffer of your choice. Use enough buffer to completely cover the blot and allow it to move freely in the dish.
4. Block membrane for 30-60 minutes at room temperature with gentle rocking.
5. Dilute ExoBrite™ HRP 1:1000 in fresh blocking buffer. If using other primary antibodies, perform a titration to find the optimal concentration. 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.
6. Incubate membrane with gentle rocking for 1-2 hours at room temperature or overnight at 4°C, protected from light.
7. 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.
8. If only using ExoBrite™ HRP labeled primary antibodies, continue to step 11. If combining HRP conjugates with unlabeled primary antibodies and fluorescent secondary antibodies, continue to step 9.
9. 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 5. Incubate membrane for 30 minutes to 2 hours with rocking.

    Note: Avoid using anti-mouse secondary antibodies because they will also bind ExoBrite™ Western Antibodies.

10. Wash membrane as in step 7.
11. Prepare a working solution of ECL substrate based on the manufacturer's protocol. Incubate the blot with the working solution for 1-5 minutes, depending on the ECL substrate used.
12. Remove the blot from the ECL substrate working solution. For detection, use x-ray film or a chemiluminescence imager, or a multimode imager for combining HRP and fluorescent conjugates.

    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.

Table 2. Detection Settings for ExoBrite™ Western Antibodies

Dye	Abs/Em	Imaging System	Excitation	Emission Filter
ExoBrite™ 680/700	681/698 nm	Amersham Typhoon™ Trio; Amersham Typhoon™ RGB	630 nm	670BP30
		Amersham Typhoon™ 5; Amersham Typhoon™ NIR	685 nm	720BP20
		Amersham Imager 680 RGB	630 nm	705BP40
		UVP ChemStudio and UVP ChemStudio PLUS	660 nm (IR1)	730 nm
		LICORbio™ Odyssey®; Odyssey® CLx; Atlas™	700 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	Far-red channel	
		Azure C500; Azure C600; Azure Sapphire Imager	660 channel	
ExoBrite™ 770/800	770/797 nm	Amersham Typhoon™ 5; Amersham Typhoon™ NIR	785 nm	825BP30
		UVP ChemStudio and UVP ChemStudio PLUS	785 nm (IR2)	810 nm
		LICORbio™ Odyssey®; Odyssey® CLx; Atlas™	800 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	NIR channel	
		Azure C500; Azure C600; Azure Sapphire Imager	785 channel	
ExoBrite™ HRP	N/A	UVP ChemStudio and UVP ChemStudio Plus	Chemiluminescence	
		ChemiDoc™ MP Imaging System (Bio-Rad)		
		Azure C280; Azure C300; Azure C400; Azure C500; Azure C600; Azure Sapphire Imager		
		LICORbio C-DiGit®; Odyssey® Fc; Odyssey® CLx; Odyssey® M; Atlas™		
		X-ray film	N/A	

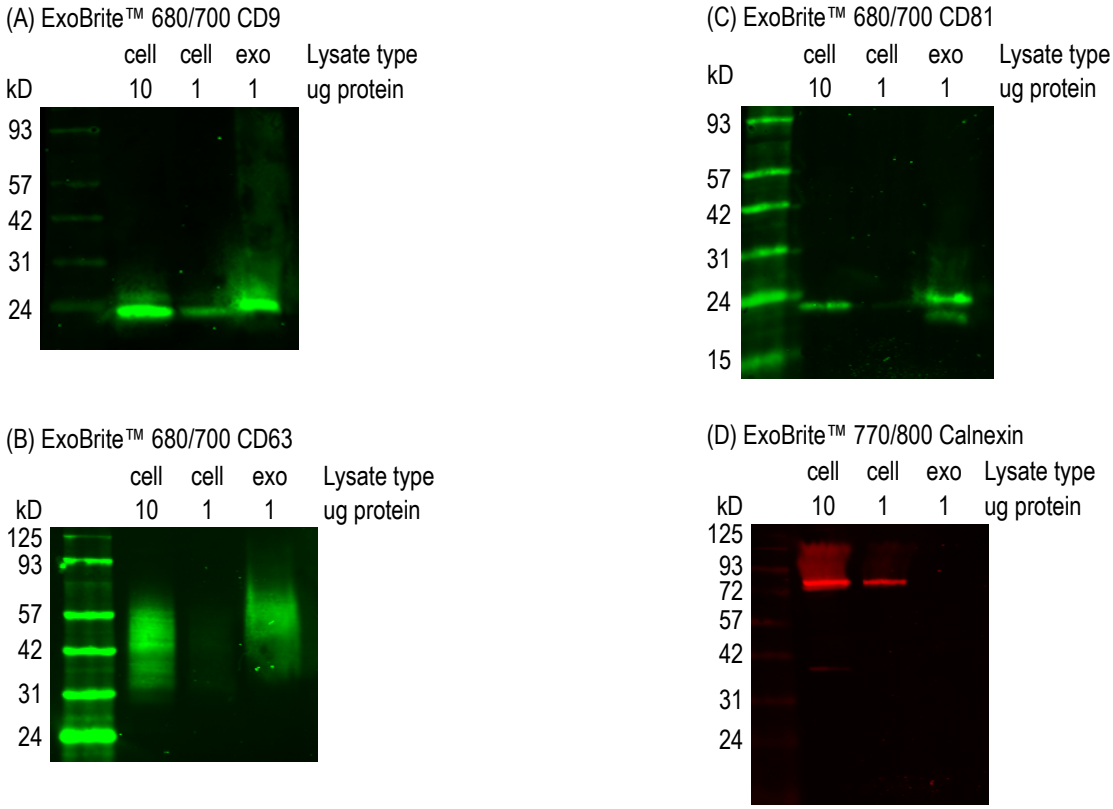

Figure 1. Western detection of human CD9, CD63, CD81, and calnexin in cell and exosome lysate using ExoBrite™ Western Antibodies, showing enrichment of the tetraspanin proteins and depletion of calnexin in the exosome prep. Exosomes were isolated from MCF-7 cell conditioned medium by size exclusion column. The indicated amounts of lysates from MCF-7 cells and MCF-7 derived vesicles were run on an acrylamide gel and transferred to PVDF. The membranes were blocked with TrueBlack® Western Blocking Buffer, and then stained with 1X ExoBrite™ Western Antibody. (A) ExoBrite™ 680/700 CD9 Western Antibody, (B) ExoBrite™ 680/700 CD63 Western Antibody, (C) ExoBrite™ 680/700 CD81 Western Antibody, or (D) ExoBrite™ 770/800 Calnexin Western Antibody. The membranes were imaged on a LICORbio™ Odyssey® infrared imaging system in the 700 channel for ExoBrite™ 680/700, or the 800 channel for ExoBrite™ 770/800. The protein ladder is Peacock™ Prestained Protein Marker, 1.5 uL per lane.

Troubleshooting Tips for Fluorescent Western Detection

Problem	Potential Causes/Diagnosis	Potential Solutions
No staining or low signal	Primary antibody not validated for application	If you are using primary antibodies other than ExoBrite™ Western Antibodies: • Check to see if the primary antibody has been validated for detection by western blot. • Check that the species reactivity of the antibody is compatible with your sample.
	No EVs in prep or target not expressed in sample	Use a total cell lysate as a positive control to confirm that the antibody works. If possible, use a cell lysate from the same cell or tissue that the EVs derive from to determine whether the protein of interest is expressed in that cell or tissue. If it is expressed in the cells but not the EV prep, we recommend troubleshooting the EV isolation procedure. Read our Tech Tip: Fluorescent Detection of EVs by Flow Cytometry for tips and a comparison of different EV isolation methods.
	Antibody concentration too low	For ExoBrite™ Western Antibodies, we recommend starting with a concentration of 1:1000. However, you may need to titrate the antibody concentration depending on your cell/EV source and protocols. If you are using other primary antibodies, we also recommend performing a titration 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 ug/mL for near-IR western detection.
	Imaging settings not compatible with dyes	Review the recommended detection settings in Table 2 to make sure 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 near-IR scanner.
High background or non-specific staining	Cross-reactivity of secondary antibody with ExoBrite™ Western Antibody Conjugates, other primary antibodies, or proteins in sample	Avoid using secondary antibodies that may cross-react with the host species of other primary antibodies. For example, anti-mouse secondary antibodies should be avoided if using ExoBrite™ Western Antibody Conjugates.
	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 regular PVDF can give higher sensitivity, possibly due to higher protein binding.
	Improper wetting of PVDF membrane	If PVDF membrane has been allowed to dry after western transfer, it must be briefly re-wet in methanol, then rinsed in water before continuing with blocking and detection.
	Suboptimal western blot blocking	Test different blocking agents to find the optimal conditions, or try a blocking buffer specifically designed for fluorescent westerns. We find that our TrueBlack® WB Blocking Buffer Kit (Cat. No. 23013) gives low background with ExoBrite™ fluorescent conjugates.
	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.	For ExoBrite™ Western Antibodies, we recommend a starting concentration of 1:1000. However, if the concentration seems too high we recommend titrating at lower concentrations to find the optimal concentration. If you are using other primary antibodies we also recommend performing a titration 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 ug/mL for near-IR western detection.
	Calnexin detected in EV samples	We recommend optimizing your EV isolation procedure if your EV sample contains calnexin. Read our Tech Tip: Fluorescent Detection of EVs by Flow Cytometry for tips and a comparison of different EV isolation methods.

Troubleshooting Tips for Chemiluminescent Western Detection

Problem	Potential Causes/Diagnosis	Potential Solutions
No staining or low signal	Primary antibody not validated for application	If you are using primary antibodies other than ExoBrite™ Western Antibodies: • Check to see if the primary antibody has been validated for detection by western blot. • Check that the species reactivity of the antibody is compatible with your sample.
	No EVs in prep or target not expressed in sample	Use a total cell lysate as a positive control to confirm that the antibody works. If possible, use a cell lysate from the same cell or tissue that the EVs derive from to determine whether the protein of interest is expressed in that cell or tissue. If it is expressed in the cells but not the EV prep, we recommend troubleshooting the EV isolation procedure. Read our Tech Tip: Fluorescent Detection of EVs by Flow Cytometry for tips and a comparison of different EV isolation methods.
	Antibody concentration too low	For ExoBrite™ Western Antibodies, we recommend starting with a concentration of 1:1000. However, you may need to titrate the antibody concentration depending on your cell/EV source and protocols. If you are using other primary antibodies, we also recommend performing a titration 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 ug/mL for near-IR western detection.
	Too much HRP If brown or yellow bands are present HRP enzyme, there is too much HRP.	The substrate is the limiting factor in chemiluminescent western blotting and using too much HRP will exhaust the substrate quickly, resulting in low signal. Also, HRP leaves brown or yellow bands on the membrane when oxidized, which is indicative of too much HRP being used. Titrate the HRP conjugate down to obtain the correct balance of enzyme and substrate.
	Too much target protein If ghost or hollow bands are present, there is too much target protein.	White bands on the blot or white halos within bands are caused by intense localized signal due to the depletion of ECL substrate in the white area. Load a lower amount of target protein on the gel or titrate the antibody concentration down.
	Underexposed film	Increase the exposure time of the membrane to the film.
High background or non-specific staining	Cross-reactivity of secondary antibody with ExoBrite™ Western Antibody Conjugates, other primary antibodies, or proteins in sample	Avoid using secondary antibodies that may cross-react with the host species of other primary antibodies. For example, anti-mouse secondary antibodies should be avoided if using ExoBrite™ Western Antibody Conjugates.
	Improper wetting of PVDF membrane	If PVDF membrane has been allowed to dry after western transfer, it must be briefly re-wet in methanol, then rinsed in water before continuing with blocking and detection.
	Suboptimal western blot blocking	Test different blocking agents to find the optimal conditions, or try a blocking buffer specifically designed for chemiluminescent westerns.
	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.	For ExoBrite™ Western Antibodies, we recommend a starting concentration of 1:1000. However, if the concentration seems too high we recommend titrating at lower concentrations to find the optimal concentration. If you are using other primary antibodies we also recommend performing a titration 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 ug/mL for near-IR western detection.
	Overexposed film	Decrease the exposure time of the membrane to the film.

Related Products

Cat. No.	Product
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
33025,33026	VersaBlot™ Total Protein Normalization Kits
23013	TrueBlack® WB Blocking Buffer Kit
22010	10X Fish Gelatin Blocking Agent
22014	Bovine Serum Albumin 30% Solution
22013	Bovine Serum Albumin Fraction V
22012	Dry Milk Powder
22011	Fish Gelatin Powder
22033	1X PBS (2L) Buffer Powder Packets
22002	Tween® 20
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA EV Staining Kits
28002	ExoBrite™ EV Stain Enhancer (100X)
P003-410... P022-APC	ExoBrite™ Flow Antibody Conjugates
P003-498ST... P022-647PST	ExoBrite™ STORM Antibodies
30129... 30137	ExoBrite™ True EV Membrane Stains
28001	ExoBrite™ EV Total RNA Isolation Kit
30127	ExoBrite™ EV Surface Stain Sampler Kit, Green
28000	ExoBrite™ Streptavidin Magnetic Beads

Please visit our website at www.biotium.com for information on our products for EV detection and western blotting, including EV stains and antibodies for flow cytometry, western blot blocking buffers, and total protein stains.