Revised: July 28, 2022

Product Information

ExoBrite™ Western Antibody Conjugates

Product List

Catalog 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
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
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
P007-770-250	ExoBrite™ 770/800 Calnexin Western Antibody	25 tests
P007-770-1000	ExoBrite™ 770/800 Calnexin Western Antibody	100 tests

Storage and Handling

Store at 4°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

Extracellular vesicles (EVs), including exosomes, are lipid-bound vesicles that are released from cells. EVs display specific surface proteins and can carry nucleic acids and other cargo, allowing them to transfer biological information between cells in different parts of the body. Therefore exosomes are increasingly studied for their potential use in drug delivery and medical diagnostic applications. The most common proteins used as exosome 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 exosomes. These tetraspanins are broadly expressed on many cell types and can therefore be detected on many types of exosomes, but their expression levels vary depending on the cell type of origin.

ExoBrite™ Western Antibody Conjugates are validated by Biotium for optimal detection of exosome markers CD9, CD63, and CD81 in isolated EV extracts by fluorescent 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 also conjugated to near-infrared (near-IR) fluorescent CF® Dyes, 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 exosomes. It is offered as a negative control to assess the purity of isolated exosome extracts.

Biotium also offers ExoBrite™ EV Membrane Staining Kits and ExoBrite™ Flow Antibody Conjugates for detection of isolated EVs and exosomes by flow cytometry (see Related Products).

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 exosome purity assessment)	IgG1, kappa	821	P27824	567968	IP90

General Considerations for Exosome Isolation for Western Blotting

- Obtaining a clean exosome prep is crucial for obtaining robust signal and proper interpretation of results. While there are several exosome 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 exosomes. For detailed protocols on exosome isolation by SEC or PEG precipitation, read our Tech Tip: Exosome Isolation and Staining Protocols. For a detailed comparison of exosome isolation methods, read our Tech Tip: Fluorescent Detection of Exosomes by Flow Cytometry.
- 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 exosome lysates may be lower than for cell lysates, because tetraspanins are enriched in exosomes. We recommend testing 1-3 ug of protein from purified exosomes as a starting point for optimization. Higher exosome protein amounts may be required depending on the target expression level in your sample.

General Considerations for Fluorescent Western Detection

- Multiplex fluorescence western detection requires an imaging system capable of detecting fluorescent dyes in multiple channels. For best results, use a gel imager or scanner specifically designed for imaging fluorescent blots. See Table 2 for recommended detection settings for common fluorescent gel imaging systems.
- Far-red or near-IR dyes such as ExoBrite™ Western Antibodies 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 (see Related Products). 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.
- 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 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™ Western Antibodies. 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.

Fluorescent Western Blotting Protocol

Materials required but not provided

For more information on required materials, see General Considerations for Fluorescent Western Detection and Related Products.

- Optional: VersaBlot™ Total Protein Normalization Kit
- TrueBlack® WB Blocking Buffer Kit or other blocking buffer
- PBS or TBS with 0.1% Tween®-20
- Peacock™ Prestained Protein Markers 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 min.)
5. Primary antibody incubation (2 hours or overnight)
6. Washes (30 min.)
7. Optional: Secondary antibody incubation (not required for labeled primary antibody) (30 min. to 2 hours)

   Note: Only if using an unlabeled primary antibody and labeled secondary antibody.
8. Optional: Washes (~30-60 min.) (required only if using secondary antibodies)
9. Dry membrane (optional stopping point)
10. Image membrane

Table 2. Detection settings for ExoBrite™ Western Antibodies

Dye	Abs / Em	Imaging System	Excitation	Emission Filter
ExoBrite™ 680/700	681 nm / 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
		LI-COR® Odyssey®; Odyssey® CLx	700 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	Far-red channel	
		Azure C500; Azure C600, Azure Sapphire Imager	660 channel	
ExoBrite™ 770/800	770 nm / 797 nm	Amersham Typhoon™ 5; Amersham Typhoon™ NIR	785 nm	825BP30
		UVP ChemStudio and UVP ChemStudio PLUS	785 nm (IR2)	810 nm
		LI-COR® Odyssey®; Odyssey® CLx	800 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	NIR channel	
		Azure C500; Azure C600, Azure Sapphire Imager	785 channel	

Detailed protocol

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 (see Related Products).
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 (see Related Products).

   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 (see Related Products).
6. Block membrane for 30 minutes to 1 hour at room temperature with gentle rocking.
7. Dilute ExoBrite™ Western Antibody 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 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 exosome 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.

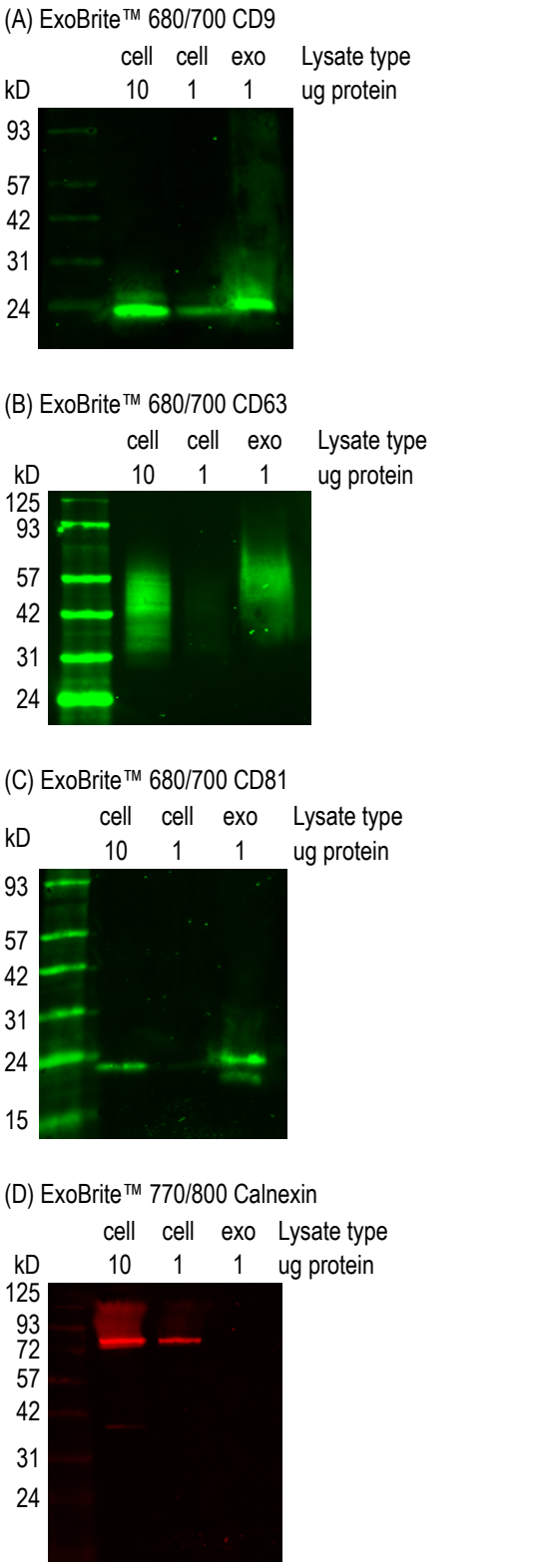

Figure 1. Western detection of human CD9, CD63, CD81, and calnexin in cell and exosome lysate using ExoBrite™ Western Antibodies, showing enrichment of the tetraspanin proteins and depletion of calnexin in the exosome prep. Exosomes were isolated from MCF-7 cell conditioned medium by size exclusion column. 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 LI-COR 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 in your western blot 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 exosomes 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 exosomes 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 exosome prep, we recommend troubleshooting the exosome isolation procedure. Read our Tech Tip: Fluorescent Detection of Exosomes by Flow Cytometry for tips and a comparison of different exosome 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/exosome 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 or 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 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 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 gives low background with ExoBrite™ Western Antibodies (see Related Products).
	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.	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 exosome samples	We recommend optimizing your exosome isolation procedure if your exosome sample contains calnexin. Read our Tech Tip: Fluorescent Detection of Exosomes by Flow Cytometry for tips and a comparison of different exosome isolation methods.

Related Products

Catalog number	Product
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
30111-30114	ExoBrite™ EV Membrane Staining Kits
P003-410... P008-RPE	ExoBrite™ Flow Antibody Conjugates
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
22002	Tween®-20

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