SZABO SCANDIC

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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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Product Information

ExoBrite™ Streptavidin Magnetic Beads

Catalog Number: 28000

Unit Size: 5 mL, 2 x 10^7 beads/mL

Storage and Handling

Store at 4°C. Do not freeze. Product is stable for at least 12 months from date of receipt when stored as 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, EVs are increasingly studied for their potential use in drug delivery and medical diagnostic applications. ExoBrite™ Streptavidin Magnetic Beads can be combined with a biotinylated antibody of your choice (not included) for capture of EVs from cell culture medium or other biological fluids. Antibodies against membrane surface markers that are enriched in EVs, such as tetraspanins (CD9, CD63, or CD81), are commonly used for this method of EV isolation. The bead-bound EVs may then be stained with antibody conjugates or other probes for detection using flow cytometry or fluorescence microscopy. The bead-bound EVs may also be lysed for analysis of proteins or nucleic acids.

Biotium offers several EV staining kits that are optimized for bright, sensitive staining of EVs and that can also be combined with antibody staining. This includes ExoBrite™ CTB EV Stains (cholera toxin subunit B), ExoBrite™ Annexin V EV Stains, and ExoBrite™ WGA EV Stains (wheat germ agglutinin). Biotium also offers a selection of fluorescent ExoBrite™ Flow Antibodies against CD9, CD63, and CD81 for multiparameter analysis of free or bead-bound EVs by flow cytometry (see Related Products).

Experimental Protocols

1. Antibody binding to streptavidin beads

This protocol provides instructions for binding biotinylated antibody to 500 uL of beads, which is enough for up to 25 EV isolation reactions using part 2 of the protocol for EV isolation. Volumes can be scaled up or down as needed.

Materials required but not provided

- Biotinylated antibody of choice
- Magnet for microcentrifuge tubes
- End over end (rotating) mixer
- Wash buffer of your choice (Recommended: 1X PBS + 0.1% BSA)
- Sodium azide

1.1 Mix the ExoBrite™ Streptavidin Magnetic Beads well by vortexing and inverting the bottle. Ensure that the beads are completely homogenous.

1.2 Aliquot 500 uL of beads to a 1.5 mL microcentrifuge tube.

1.3 Add 2 ug of biotinylated antibody to the resuspended beads. Vortex gently to mix.

Note: Amount of antibody can be optimized. We recommend an antibody range of 0.5 to 2 ug.

1.4 Incubate for 1 hour at room temperature on the end over end mixer.

1.5 Place the tube containing beads on the magnet for 1 minute. While the tube is on the magnet, carefully remove the supernatant without disrupting the beads.

1.6 Remove the tube containing beads from the magnet and resuspend the beads in 500 uL wash buffer. Vortex well to mix.

1.7 Remove the supernatant as in step 1.5. Resuspend the beads in 500 uL wash buffer. To store the antibody-bound beads for later use, add sodium azide to a final concentration of 0.02%; the beads will be stable at 4°C for at least 3 months.

2. EV isolation with antibody-bound streptavidin beads

This protocol provides instructions for isolating EVs from biological fluids such as cell culture medium. EVs are isolated using 20 uL of antibody-bound ExoBrite™ Streptavidin Magnetic Beads which is sufficient for three flow cytometry staining reactions. These are intended as guidelines; you may need to adjust the amount of beads or sample volume for your specific sample type.

Materials required but not provided

- Antibody-bound beads prepared according to part 1 of the protocol
- Magnet for microcentrifuge tubes
- End over end (rotating) mixer
- Wash buffer of your choice (Recommended: 1X PBS + 0.1% BSA)

2.1 Mix the tube containing antibody-bound ExoBrite™ Streptavidin Magnetic Beads well by vortexing and inverting. Ensure that the beads are completely homogenous.

2.2 Aliquot 20 uL of antibody-bound beads into microcentrifuge tubes, one tube per EV sample to be isolated.

Note: Each isolation using 20 uL of antibody-bound beads is sufficient for three flow cytometry staining reactions. The amount of beads per isolation can be optimized.

2.3 Spin the beads in a mini centrifuge for ~ 5 seconds to collect the beads in the bottom of the tube, then place the tube on the magnet for 1 minute. While the tubes are on the magnet, carefully remove the supernatant without disrupting the beads.

2.4 Remove the tubes from the magnet and add your EV-containing sample. Below are some examples of sample types and volumes:

a. 100 uL of conditioned medium (equivalent to 4 mL of serum-free cell culture supernatant, concentrated down to 100 uL).

b. 200 uL of PEG-precipitated enriched EVs (equivalent to 4 mL of serum-free cell culture supernatant, precipitated overnight with 2 mL of 30% PEG, and resuspended in 200 uL of PBS or buffer of choice).

2.5 Incubate the beads and sample for 1 hour at room temperature or 4°C on the end over end mixer.

2.6 Repeat step 2.3.

2.7 Wash the beads with 300 uL of wash buffer as in step 2.4.

2.8 Repeat step 2.3.

2.9 Remove the tubes from the magnet and resuspend the bead-bound EVs in 300 uL of wash buffer.

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2.10 At this point the bead-bound EVs are ready to be used for the desired downstream application:

a. For fluorescence staining with ExoBrite™ EV Stains and/or ExoBrite™ Flow Antibodies for analysis by flow cytometry, see the relevant product information sheets for protocols.

b. For western blotting analysis, the beads can be directly suspended in 1X RIPA buffer without reducing agent. The beads can then be removed using the magnet and the supernatant can be loaded onto the gel. For ultra-sensitive fluorescent western, membranes can be probed with ExoBrite™ Western Blot Antibodies. See product information sheets for protocols.

Note: Suspending the beads with lysis buffers other than RIPA buffer and/or reducing agents can cause the antibody and streptavidin monomers to separate from the beads and affect downstream analysis.

Related Products

Cat. No.	Product
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
P003-410	ExoBrite™ 410/450 CD9 Flow Antibody
P003-490	ExoBrite™ 490/515 CD9 Flow Antibody
P003-560	ExoBrite™ 560/585 CD9 Flow Antibody
P003-650	ExoBrite™ 650/665 CD9 Flow Antibody
P003-RPE	ExoBrite™ R-PE CD9 Flow Antibody
P004-410	ExoBrite™ 410/450 CD63 Flow Antibody
P004-490	ExoBrite™ 490/515 CD63 Flow Antibody
P004-560	ExoBrite™ 560/585 CD63 Flow Antibody
P004-RPE	ExoBrite™ R-PE CD63 Flow Antibody
P005-410	ExoBrite™ 410/450 CD81 Flow Antibody
P005-490	ExoBrite™ 490/515 CD81 Flow Antibody
P005-560	ExoBrite™ 560/585 CD81 Flow Antibody
P005-RPE	ExoBrite™ R-PE CD81 Flow Antibody
P008-410	ExoBrite™ 410/450 IgG1 Isotype Control Flow Antibody
P008-490	ExoBrite™ 490/515 IgG1 Isotype Control Flow Antibody
P008-560	ExoBrite™ 560/585 IgG1 Isotype Control Flow Antibody
P008-650	ExoBrite™ 650/665 IgG1 Isotype Control Flow Antibody
P008-RPE	ExoBrite™ R-PE IgG1 Isotype Control Flow Antibody
P003-680	ExoBrite™ 680/700 CD9 Western Antibody
P003-770	ExoBrite™ 770/800 CD9 Western Antibody
P004-680	ExoBrite™ 680/700 CD63 Western Antibody
P004-770	ExoBrite™ 770/800 CD63 Western Antibody
P006-680	ExoBrite™ 680/700 CD81 Western Antibody
P006-770	ExoBrite™ 770/800 CD81 Western Antibody
P007-770	ExoBrite™ 770/800 Calnexin Western Antibody

Please visit our website at www.biotium.com for more 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.