ExoBacteria™ OMV Isolation Kit (for *E.coli* and other gram-negative bacteria)

Cat # EXOBAC100A-1

User Manual

Storage: Please see individual components

Version 2
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Contents

Product Description

Bacteria-derived outer membrane vesicles (OMVs for short) have recently gained attention as a novel mechanism for transmission of molecular information within bacteria as well as bacteria to mammalian cells. Owing to its similarities to well-established extracellular vesicles (EVs), researchers have utilized OMVs to study host-bacterial interactions in pathogenesis[1], in cancer therapy[2], in modulating host immune response[3], and engineering for use as vaccines[4].

To assist researchers in identifying new and exciting applications for OMVs, SBI is proud to introduce the ExoBacteria™ OMV Isolation Kit – the first isolation system dedicated for OMV isolation (from *E.coli* and other Gram-negative species, e.g. *P. putida*) for use in downstream applications. Using an innovative precipitation-free, ion-exchange chromatography system (containing a proprietary capture resin + gravity column) for capture of OMVs from bacterial culture medium, the kit delivers OMVs in less than 1 hr of total time with purity and yield rivaling ultracentrifugation-based approaches.

The kits are provided with enough reagents and consumables to process up to 20 reactions*, allowing researchers maximal flexibility with experimental conditions.

***1 reaction is defined as 1 ml of resin + 30 ml of purified bacterial culture media loaded into a single gravity column**

List of Components

Item	Volume/Qty	Storage Temperature
OMV Binding Resin	20 mL	4°C
Binding Buffer (20X)	60 mL	4°C
Elution Buffer (1X)	30 mL	RT
Gravity Flow Columns	20	RT
Column Stoppers	20	RT
Column Caps	20	RT

Storage

The Kit is shipped on Blue Ice and the components should be stored at recommended temperatures as stated above. Properly stored kits are stable for 12 months from the date received.

General Information

Note: The Binding Buffer is provided in a 20X concentration, please dilute to a 1X working concentration in DI water prior to starting the procedure.

Materials required but not provided: Filtering units with 0.45 µm and 0.22 µm filters, column rack, rotating rack, pipettes and pipette tips. For column racks, we would suggest QIArack (Cat #10915) from Qiagen for holding the columns and collection of flow-through material.

Protocol for ExoBacteria OMV Isolation Kit:

1. **Prepare clarified supernatant from bacterial culture**
    a. Culture the bacteria in its appropriate condition until the desired OD is reached.
    b. Spin down bacteria at 5000 x g for 20 mins at 4°C.
    c. Transfer the supernatant to a fresh tube and spin again at 5000 x g for 20 mins at 4°C to completely remove any cellular debris.
    d. Collect the culture supernatant and filter through a 0.45 µm vacuum filter.
    e. Repeat the filtration step with a 0.22 µm vacuum filter and collect the material.

    Filtered culture supernatant is now ready for OMV isolation.

2. **Column preparation and OMV capture**
    a. Mix the OMV Binding Resin by shaking the bottle thoroughly as contents may have settled at the bottom
    b. Pipette 1 mL of the OMV Binding Resin onto the column.
    c. Equilibrate by adding 10 mL of the 1X Binding Buffer to column and allow solution to completely flow through. Discard the flow through.
    d. Place a yellow column stopper onto the bottom of the column.
    e. Add 30 mL of the clarified bacterial culture supernatant (prepared in step 1) to the resin and place column cap on top of the column.
    f. Incubate on a rotating rack at 4°C for 30 minutes to allow for OMV binding

3. **OMV Elution**
    a. Place the column onto a rack and uncap the top and bottom of the column
    b. Allow the resin/supernatant mixture to flow through
    c. Wash the resin with 15 mL Binding Buffer. Discard the flow through. Repeat the wash step 2x.
    d. Cap the column at the bottom with the stopper and add 1.5 mL OMV Elution Buffer. Incubate for 2 mins at room temperature, gently shaking the column every 30 secs.
    e. Uncap the column at the bottom and collect the eluate containing the OMVs in a fresh microcentrifuge tube.

Example Data and Applications

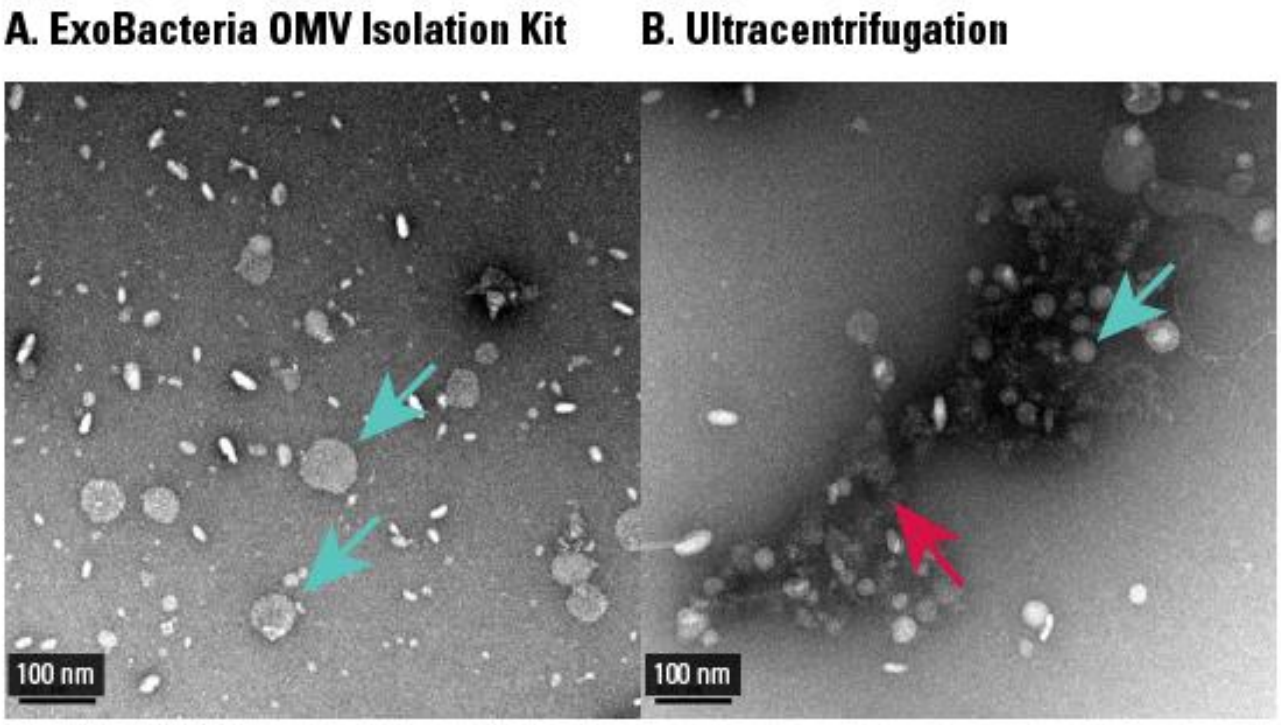
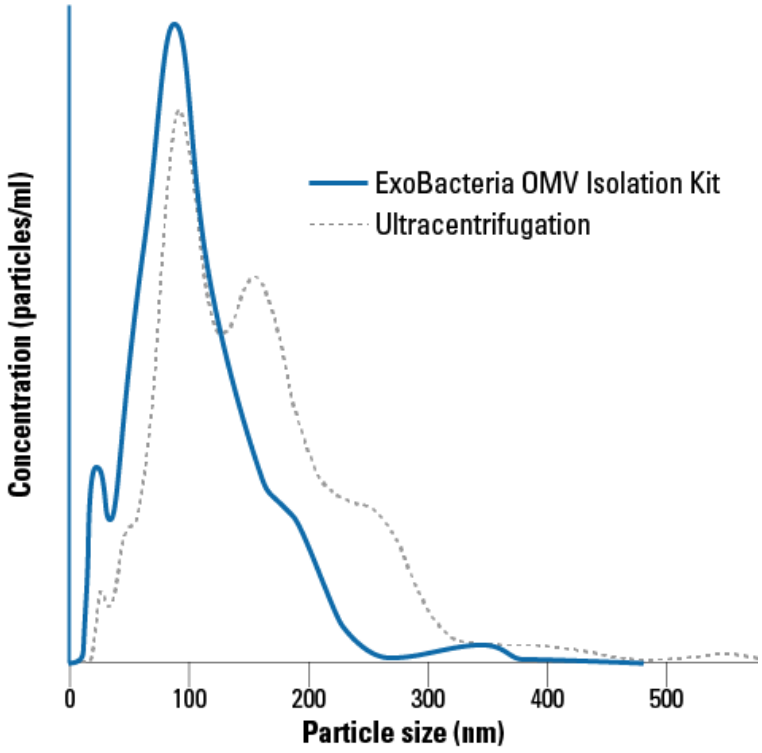


Figure 1. OMVs isolated using the ExoBacteria OMV Isolation Kit are similar in appearance, but with tighter distribution than OMVs isolated via ultracentrifugation. Comparison of transmission electron micrographs (TEM) of E. coli-derived OMVs isolated using **(A)** the ExoBacteria OMV Isolation Kit or **(B)** ultracentrifugation. OMVs (light blue arrows) are indicated in both samples, but note the unwanted protein aggregates (red arrow) that appear in the ultracentrifugation sample.

A. Particle size distribution



B. Yield

	OMV Yield (particles/µg protein)
ExoBacteria OMV Isolation Kit	1.38×10^8
Ultracentrifugation	0.0657×10^8

Figure 2. The ExoBacteria OMV Isolation Kit delivers a narrower size distribution of OMVs and higher yields than ultracentrifugation. (A) Comparison of fluorescent NTA analysis of E. coli-derived bacterial OMVs isolated using either ExoBacteria OMV Isolation Kit (blue line) or ultracentrifugation (gray line) shows how the ExoBacteria OMV Isolation Kit delivers a much narrower size distribution of isolated OMVs than conventional ultracentrifugation approach. **(B)** In addition, total yield is 20-fold higher with the ExoBacteria OMV Isolation Kit (1.38 x 10^8 particles/µg of protein) compared to ultracentrifugation (0.0657 x10^8 particles/µg of protein).

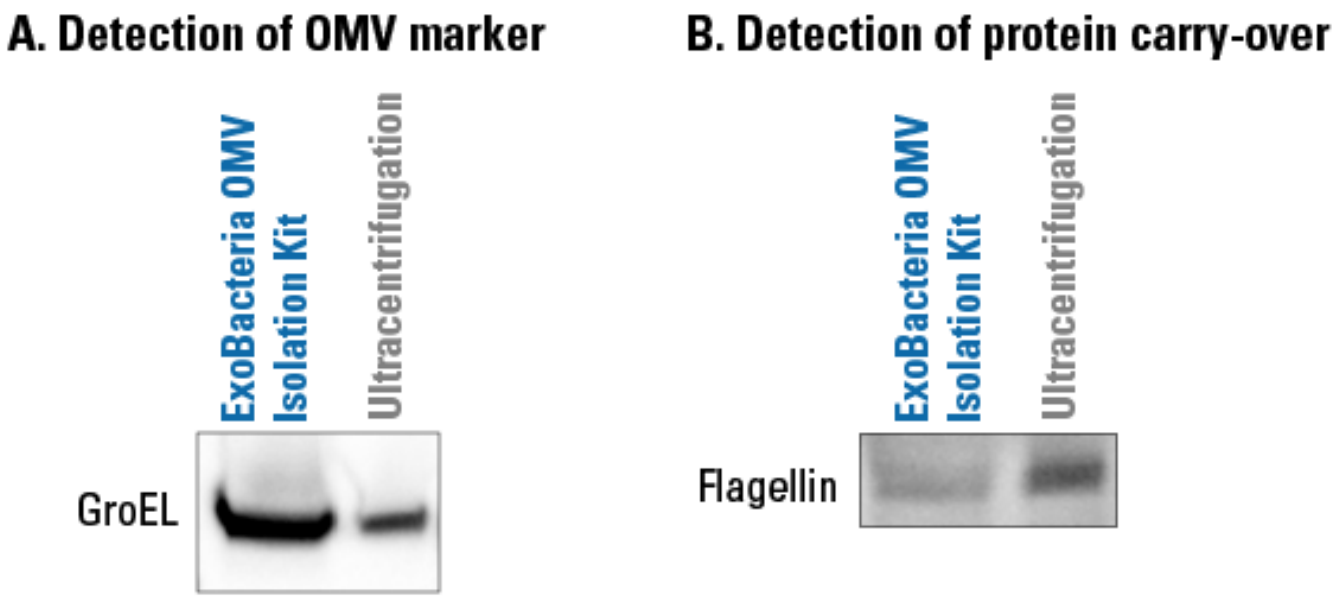


Figure 3. Get higher OMV yield and less carry-over protein with the ExoBacteria OMV Isolation Kit. Western blot analyses of E. coli-derived OMVs isolated using either the ExoBacteria OMV Isolation Kit or ultracentrifugation show that **(A)** the ExoBacteria OMV Isolation Kit delivers higher yield of OMVs than ultracentrifugation—compare the amounts of GroEL in each lane—and **(B)** the ExoBacteria OMV Isolation Kit preps have less carry-over protein than the ultracentrifugation preps—note the lower amount of flagellin. Each lane is loaded with 5 µg of total protein.

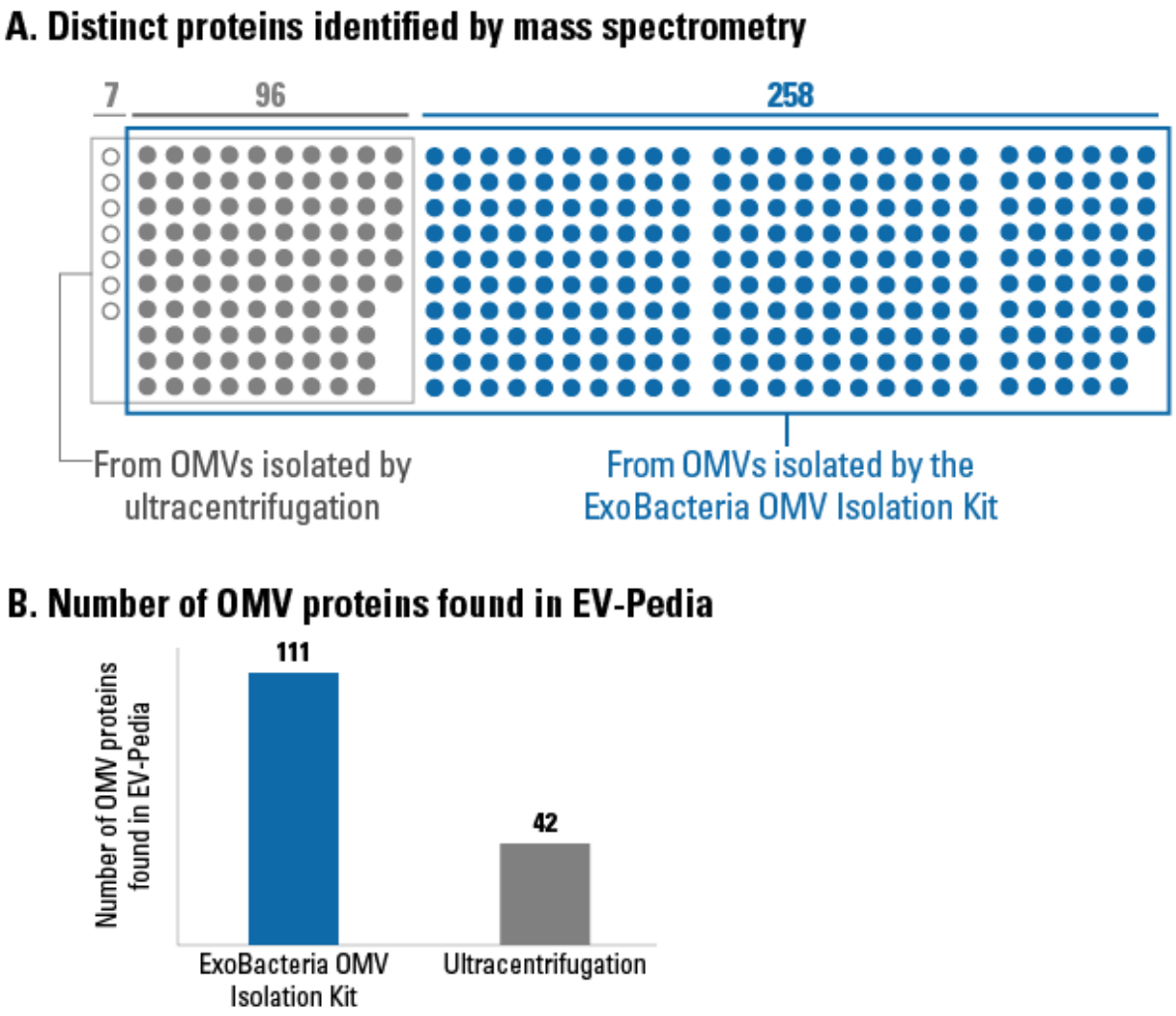


Figure 4. Identify more OMV proteins with OMVs isolated using the ExoBacteria OMV Isolation Kit. (A) Comparison of the total number of proteins identified via mass spectrometry from OMV samples isolated using either the ExoBacteria OMV Isolation Kit or ultracentrifugation shows a wider range of proteins identified from OMVs isolated with the ExoBacteria OMV Isolation Kit. All but 7 of the proteins identified in the OMVs isolated using ultracentrifugation were also found in the OMVs isolated using the ExoBacteria kit, suggesting that the higher yield and higher purity delivered by the ExoBacteria OMV Isolation Kit expand your ability to derive insights from OMVs. **(B)** More of the proteins from the ExoBacteria OMV kit-isolated OMVs were found in the EV-Pedia database than from OMVs isolated using ultracentrifugation, demonstrating the increased specificity of the ExoBacteria OMV Isolation Kit.

References

1. Vanaja SK *et al.* Bacterial Outer Membrane Vesicles Mediate Cytosolic Localization of LPS and Caspase-11 Activation. Cell 2016 May 19; 165(5): 1106-1119.

2. Kim OY *et al.* Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response. Nat Commun. 2017 Sep 20;8(1):626. doi: 10.1038/s41467-017-00729-8

3. Vidakovics ML *et al.* B cell activation by outer membrane vesicles--a novel virulence mechanism. PLoS Pathog. 2010 Jan 15;6(1):e1000724. doi: 10.1371/journal.ppat.1000724.

4. Chen DJ et al. Delivery of foreign antigens by engineered outer membrane vesicle vaccines. Proc Natl Acad Sci U S A. 2010 Feb 16;107(7):3099-104. doi: 10.1073/pnas.0805532107. Epub 2010 Jan 27.

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