Preparation of Outer Membrane Vesicle from *Escherichia coli*

Oh Youn Kim, Bok Sil Hong, Kyong-Su Park, Yae Jin Yoon, Seng Jin Choi, Won Hee Lee, Tae-Young Roh, Yoon-Keun Kim and Yong Song Gho*

Life Sciences Department, Pohang University of Science and Technology (POSTECH), Pohang, Republic of Korea

*For correspondence: ysgho@postech.ac.kr

[Abstract] Outer membrane vesicles (OMVs) are spherical bilayered phospholipids of 20-200 nm in size produced from all Gram-negative bacteria and Gram-positive bacteria investigated to date. Previous biochemical and proteomic studies have revealed that the Gram-negative bacteria-derived OMVs are composed of various components like outer membrane proteins, lipopolysaccharides, outer membrane lipids, periplasmic proteins, DNA, and RNA. Here, in this protocol, we describe the method to isolate the OMVs from the culture supernatant of *Escherichia coli (E. coli)*.

Materials and Reagents

1. Phosphate buffered saline (PBS) (Gibco®, catalog number: 70013-032)
2. *E. coli* (Isolated from the peritoneal lavage fluid of cecal ligation and puncture-operated mice)
3. Luria-Bertani broth (LB) medium (Merck KGaA, catalog number: 1.10285.0500) (see Recipes)

Equipment

1. 2 L Flasks
2. Shaking incubator
3. Centrifuge
4. 500 ml Bottle top filter 43 mm neck (0.45 µm and 0.22 µm) (Corning, catalog number: 430514, 430513)
5. QuixStand Benchtop System (Amersham Biosciences, catalog number: 56-4107-44)
6. 100-kDa hollow-fiber membrane (Amersham Biosciences, catalog number: 56-4101-33)
7. Vacuum pump

   Note: All centrifuge tubes and flasks should be autoclaved before use to avoid contamination.

Procedure

1. A single colony of *E. coli* is transferred to 5 ml of LB broth.
2. The bacteria are incubated in an orbital bacteria shaking incubator at 200 rpm at 37 °C overnight (8 h).
3. LB broth of 500 ml is inoculated with 1/100 volume of the overnight cultured cells.
   Notes:
   a. *Use 2 L flask when culturing 500 ml. Also, since 1/100 volume of 5 ml is 50 µl, before inoculation, increase the volume by adding about 900 µl of fresh LB medium to reduce cell loss.*
   b. *The yield of OMVs in terms of protein amount is 100 µg per 1 liter of E. coli culture.*
4. The cells are grown for 12 h at 200 rpm at 37 °C.
5. The cells are pelleted at 5,000 *x g* for 15 min.
6. The supernatant fraction is collected and pelleted again at 5,000 *x g* for 15 min.
7. The supernatant is collected and filtered through a bottle top filter of pore size 0.45 µm using a vacuum pump.
8. The filtered supernatant is concentrated to 50-fold by ultra-filtration with a Quixstand Benchtop System using a 100 kDa hollow-fiber membrane.
   Note: Because the yield of OMVs is very low, in order to obtain a visible pellet after ultracentrifugation, the total volume of bacteria culture should be more than 5-7 liters. However, depending on the amount of OMV needed, the volume of bacteria culture could be reduced as well as the degree of concentration. Ex. 7 L of bacteria culture supernatant is concentrated to give about 280 ml of the concentrated supernatant to be filled in the total of four ultra-centrifuge tube (70 ml each).
9. The concentrated supernatant is filtered once again through a 0.22 µm vacuum filter to remove any remaining debris or bacteria.
10. The resulting filtrate is subjected to ultra-centrifugation at 150,000 *x g* for 3 h at 4 °C.

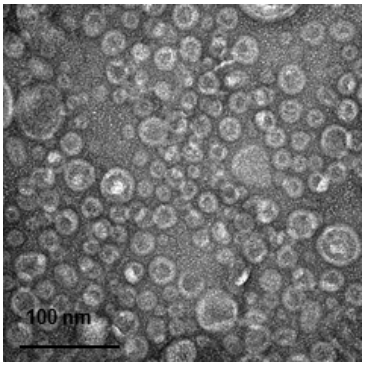

Figure 1. TEM image of *E. coli* OMV

11. The supernatant is removed and the pellet (purified OMV) is resuspended in PBS and stored at -80 °C until use.

Recipes

1. LB medium
   1% Tryptone, 0.5% yeast extract, 200 mM NaCl

Acknowledgments

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