A protocol for exosome detection via the ultrafast-isolation system (EXODUS)

Yuchao Chen
School of Ophthalmology and Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China

Qingfu Zhu
School of Ophthalmology and Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China

Luke P. Lee (lplee@bwh.harvard.edu)
Department of Medicine, Division of Engineering in Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, United States https://orcid.org/0000-0001-9663-3109

Fei Liu (feiliu@wmu.edu.cn)
School of Ophthalmology and Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou, Zhejiang 325035, China https://orcid.org/0000-0001-5259-5753

Method Article

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Abstract

Here we present a protocol for isolation and purification of exosomes using exosome detection via the ultrafast-isolation system (EXODUS). This protocol is associated with our Nature Methods paper: Exosome Detection via the Ultrafast-isolation System: EXODUS.

Introduction

Exosomes are membrane-enclosed vesicles with a size range of 30-150 nm that can be released by almost all cell types. Since exosomes are involved in cell-to-cell communication, cellular regulation, and the development of diseases, exosomes have been isolated from different biofluids such as plasma, urine, and saliva for effective investigation of various exosomal cargos. However, exosomes based biological investigation and clinical translation remain challenging due to the complexity of biological fluidics and technical limitations during exosome sample preparation. This protocol describes an automatic exosome purification method using EXODUS that has the capability to isolate EVs from different biofluids with a significantly improved throughput, stability, exosome yield, and purity. The method will include the operation and the maintenance of the EXODUS system and the procession of different biofluids. All relevant data of our experiments are detailed in a manuscript submitted to *Nature Methods* for publication (Y. Chen, *et.al*, Exosome Detection via the Ultrafast-isolation System: EXODUS, Nature Methods).

Reagents

DMEM (11960044, Gibco)

Premium Grade Fetal Bovine Serum (FBS) (10091148, Gibco)

Penicillin-Streptomycin Solution, 100X (10378016, Gibco)

Sterile PBS (C10010500CP, Gibco)

TrypLE (15400054, Gibco)

DMSO (S-002-D, Sigma-Aldrich)

EDTA (798681, Sigma-Aldrich)

Equipment

Centrifuge (5424R, 5804R, Eppendorf)

Nanoparticle Tracking Analyzer (NS300, Malvern Panalytical)

T-25 cell culture flask (430639 Corning)

CO_2 incubator (3111, Thermo Fisher)

Salivette® (51.1534, SARSTEDT)

15 mL tube (430790, Corning)

1.5 mL centrifugation tube (MCT-150-C-S, Axygen)

0.22 μm syringe filter (FPE204013, FPE204030, BIOFIL)

10 mL syringe (301945, BD)

EXODUS system

Procedure

Case 1: Isolation and purification of exosomes from urine samples:

Step 1: Collect a urine sample in a 15 mL tube (10 mL).

Step 2: Centrifuge at 2,000 x g, 4 °C for 10 min and transfer the supernatants to a new tube.

Step 3: Filter supernatant with a 0.22 μm syringe filter and collect filtration in a 15 mL tube.

Step 4: Start the EXODUS system and load the prepared sample tube in the sample holder.

Step 5: Set the isolation parameters, Negative pressure: -30 kPa, Conversion time: 10 s, Sample wash times: 2.

Step 6: Press the button “Out” to load the EXODUS device

Step 7: Press the button “Start Analyzing” to start exosome isolation. The EXODUS device will come out from the machine automatically after sample analysis.

Step 8: Use a pipette to collect the exosome solution from the EXODUS device and reconstitute to 300 μL in a 1.5 mL centrifugation tube with 1X PBS.

Case 2: Isolation and purification of exosomes from cell culture medium:

Step 1: Seed 1 x 10^6 293t cells per T-25 flask in 8 mL DMEM with 10% FBS.

Step 2: After 24h, remove the medium and wash with PBS and then change the medium to FBS free DMEM medium.

Step 3: After 24h, collect the cell culture supernatant (15 mL).

Step 4: Centrifuge at 1,000 x g, 4 °C for 10 min and transfer the supernatants to a new tube (14 mL).

Step 5: Filter supernatant with a 0.22 μm syringe filter and collect filtration in a 15 mL tube.

Step 6: Start the EXODUS system and load the prepared sample tube in the sample holder.

Step 7: Set the isolation parameters, Negative pressure: -20 kPa, Conversion time: 10 s, Sample wash times: 2.

Step 8: Press the button “Out” to load the EXODUS device

Step 9: Press the button “Start Analyzing” to start exosome isolation. The EXODUS device will come out from the machine automatically after sample analysis.

Step 10: Use a pipette to collect the exosome solution from the EXODUS device and reconstitute to 300 μL in a 1.5 mL centrifugation tube with 1X PBS.

Case 3: Isolation and purification of exosomes from human saliva:

Step 1: Collect 1 mL of human saliva with Salivette®.

Step 2: Centrifuge at 2,000 x g, 4 °C for 10 min, and transfer the supernatants to a new tube.

Step 3: Filter supernatant with a 0.22 μm syringe filter and make a 5x dilution with PBS in a 15 mL tube.

Step 4: Start the EXODUS system and load the prepared sample tube in the sample holder.

Step 5: Set the isolation parameters, Negative pressure: -30 kPa, Conversion time: 10 s, Sample wash times: 2.

Step 6: Press the button “Out” to load the EXODUS device

Step 7: Press the button “Start Analyzing” to start exosome isolation. The EXODUS device will come out from the machine automatically after sample analysis.

Step 8: Use a pipette to collect the exosome solution from the EXODUS device and reconstitute to 300 μL in a 1.5 mL centrifugation tube with 1X PBS.

Case 4: Isolation and purification of exosomes from plasma samples:

Step 1: Take 20 mL plasma sample and dilute to a final volume of 1 mL with PBS in a 15 mL tube (15 mL).

Step 2: Filter the sample with a 0.22 μm syringe filter and collect filtration in a 15 mL tube.

Step 3: Start the EXODUS system and load the prepared sample tube in the sample holder.

Step 4: Set the isolation parameters, Negative pressure: -30 kPa, Conversion time: 30 s, Sample wash times: 2.

Step 5: Press the button “Out” to load the EXODUS device

Step 6: Press the button “Start Analyzing” to start exosome isolation. The EXODUS device will come out from the machine automatically after sample analysis.

Step 7: Use a pipette to collect the exosome solution from the EXODUS device and reconstitute to 300 μL in a 1.5 mL centrifugation tube with 1X PBS.

Case 5: Isolation and purification of exosomes from tear samples:

Step 1: Collect human tears with Schirmer's test paper (30 mm).

Step 2: Dissolve the test paper into 5 mL PBS and incubate on a shaker at 4 °C for 30 min.

Step 3: Centrifuge at 200 x g, 4 °C for 10 min, and 3,000 x g, 4 °C for 10 min and transfer the supernatants to a new tube (4.5 mL).

Step 4: Filter supernatant with a 0.22 μm syringe filter and collect filtration in a 15 mL tube.

Step 5: Start the EXODUS system and load the prepared sample tube in the sample holder.

Step 6: Set the isolation parameters, Negative pressure: -20 kPa, Conversion time: 20 s, Sample wash times: 2.

Step 7: Press the button “Out” to load the EXODUS device

Step 8: Press the button “Start Analyzing” to start exosome isolation. The EXODUS device will come out from the machine automatically after sample analysis.

Step 9: Use a pipette to collect the exosome solution from the EXODUS device and reconstitute to 300 μL in a 1.5 mL centrifugation tube with 1X PBS.

Troubleshooting

1. The water or detergent level is not detected

Solution: Add more water or detergent. Make sure the container has at least 30% water or detergent in volume.

2. The sample injection needle is not in the right position

Solution: Go to the operation screen and hit “Solve Problem”.

3. After sample processing, a low quantity or no exosome was collected.

Solution: Check the sample quantity and the leakage of the EXODUS device.

Time Taken

1. For isolation of exosome from urine and saliva, the sample preparation time is about 10 min for a urine sample, 15 min for a saliva sample, and exosome isolation and purification time is about 15 min.

2. For isolation of exosomes from the cell culture medium, 3 days for cell culture, and the sample preparation time is 10 min, exosome isolation and purification time around 10 min.

3. For isolation plasma sample, exosome isolation, and purification time is around 30 min.

4. For tear sample, exosome isolation and purification time is around 10 min.

Anticipated Results

The EXODUS system provides a high yield and high purity exosome product. For processing different biofluids, different sample preparation methods and different isolation parameters are needed as described in procedures. Using a nanoparticle tracking analyzer to measure the size distribution and concentration of samples, we found the sizes of exosomes for different biofluids were in a range of 30 to 200 nm, except for tear exosomes, which was in a range of 70 to 400 nm. The concentrations were 1.4 x 10^9, 5.1 x 10^8, 8.4 x 10^9, 2.8 x 10^{12}, 3.1 x 10^8 particles per milliliter, for urine, culture medium, saliva, plasma and tear samples, respectively.

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