

Isolation and Purification of Bacterial Extracellular Vesicles from Human Feces Using Density Gradient Centrifugation

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Materials

Name	Company	Catalog Number	Comments
1 % (w/v) glutaraldehyde (prepared from 2.5 % stock solution in deionized water)	ACMEC	AP1126	Morphological observation for BEVs using TEM at Step 8.3.3
1 % (w/v) methylcellulose (prepared from original powder in deionized water)	Sigma-Aldrich	M7027	Morphological observation for BEVs using TEM at Step 8.3.6
1.5 % (w/v) uranyl acetate (prepared from original powder in deionized water)	Polysciences	21447-25	Morphological observation for BEVs using TEM at Step 8.3.5
1000 μ L, 200 μ L, 10 μ L Pipette	KIRGEN	KG1313, KG1212, KG1011	Transfer the solution
5 % (w/v) bovine serum albumin solution (prepared from the original powder in TBST buffer)	Fdbio science	FD0030	Used in western blotting for blocking at Step 8.5.6
5 \times loading buffer	Fdbio science	FD006	Used in western blotting and Coomassie brilliant blue stain at Step 8.5.1
75 % (v/v) alcohol	LIRCON	LIRCON-500 mL	Surface disinfection
96-well plate	Rar	A8096	Measure the absorbance values
Anti-Calnexin antibody	Abcam	ab92573	Western blotting (Primary Antibody)
Anti-CD63 antibody	Abcam	ab134045	Western blotting (Primary Antibody)
Anti-CD9 antibody	Abcam	ab236630	Western blotting (Primary Antibody)
Anti-Flagellin antibody	Sino Biological	40067-MM06	Western blotting (Primary Antibody)
Anti-Integrin beta 1 antibody	Abcam	ab30394	Western blotting (Primary Antibody)
Anti-LPS antibody	Thermo Fisher	MA1-83152	Western blotting (Primary Antibody)
Anti-LTA antibody	Thermo Fisher	MA1-7402	Western blotting (Primary Antibody)
Anti-OmpA antibody	CUSABIO	CSB-PA359226ZA01EOD, https://www.cusabio.com/	Western blotting (Primary Antibody)
Anti-Syntenin antibody	Abcam	ab133267	Western blotting (Primary Antibody)

Anti-TSG101 antibody	Abcam	ab125011	Western blotting (Primary Antibody)
Autoclave	ZEALWAY	GR110DP	Sterilization for supplies and mediums used in the experiment
Balance	Mettler Toledo	AL104	Balance the tube sample-loaded with PBS
Bicinchoninic acid assay	Fdbio science	FD2001	Measure protein content of BEVs at Step 8.2
BioRender	BioRender	https://app.biorender.com	Make the schematic workflow of BEVs isolation and purification showed in Figure 1
Biosafety cabinet	Haier	HR1200- II B2	Perform the procedures about feces sample handling
Centrifuge 5810 R; Rotor F-34-6-38	Eppendorf	5805000092; 5804727002, adapter: 5804774000	Preprocess for BEVs (Step 3)
Chemiluminescence Apparatus	BIO-OI	OI600SE-MF	Used in western blotting for signal detection at Step 8.5.12
Cytation 5	BioTek	F01	Microplate detector for measuring the absorbance (Step 8.1) and fluorescence (Figure 6) values
Dil-labeled low density lipoprotein	ACMEC	AC12038	Definition of distribution of interfering components
Electrophoresis equipment	Bio-rad	1658033	Used in western blotting for protein separation and transfer at Step 8.5.2, 8.5.3, 8.5.5
Enhanced Chemiluminescence kit HRP	Fdbio science	FD8020	Used in western blotting for signal detection at Step 8.5.12
<i>Escherichia coli</i>	American Type Culture Collection	ATCC8739	Isolate BEVs as a positive control. Protocol: Dissolve 25 g of the LB powder in 1 L deionized water, and autoclave. Transfer the 800 μ L of preserved <i>Escherichia coli</i> into the medium. Cultivate at 37 °C in the incubator shaker. Then centrifuge at 3,000 \times g for 20 min at 4 °C, 12,000 \times g for 30 min at 4 °C, filter the supernatant through 0.22 μ m membrane, and perform ultra-speed centrifugation at 160,000 \times g for 70 min at 4 °C. Pellet defined as crude BEVs from <i>Escherichia coli</i> was suspended in 1.2 mL PBS (Step 3, 4).
Falcon tubes 50 mL	KIRGEN	KG2811	Preprocess for BEVs (Step 3)
Feto Protein Staining Buffer	Absci	ab.001.50	Coomassie brilliant blue staining at Step 8.5.4
Filter paper	Biosharp	BS-TFP-070B	Morphological observation for BEVs using TEM at Step 8.3 (Blotting the solution)
Formvar/Carbon supported copper grids	Sigma-Aldrich	TEM-FCF200CU50	Morphological observation for BEVs using TEM at Step 8.3
HEPES powder	Meilunbio	MB6078	Prepare iodixanol buffers with different concentrations for density gradient centrifugation
HRP AffiniPure Goat Anti-Mouse IgG (H+L)	Fdbio science	FDM007	Western blotting (Secondary Antibody)
HRP AffiniPure Goat Anti-Rabbit IgG (H+L)	Fdbio science	FDR007	Western blotting (Secondary Antibody)
Incubator shaker	Qiangwen	DHZ-L	Cultivate <i>Escherichia coli</i>
Kimwipes™ Delicate Task Wipes	Kimtech Science	34155	Wipe the inner wall of the ultracentrifuge tube at Step 4.15

LB broth	Hopebio	HB0128	Cultivate Escherichia coli
Low temperature freezer (-80 °C)	Haier	DW-86L338J	Store the samples
Methanol	Alalddin	M116118	Used in western blotting for activating PVDF membrane at Step 8.5.5
Micro tubes 1.5 mL	KIRGEN	KG2211	Recover fractions after density gradient centrifugation
Micro tubes 2 mL	KIRGEN	KG2911	Recover fractions after density gradient centrifugation
Micro tubes 5 mL	BBI	F610888-0001	Recover fractions after density gradient centrifugation
Microplate reader	Thermo Fisher	Multiskan MK3	Measure protein content of BEVs at Step 8.2
Millipore filter 0.22 µm	Merck millipore	SLGP033RB	Filtration sterilization; Material: polyethersulfone, PES
NaCl	GHTECH	1.01307.040	Density gradient centrifugation solution
NaOH	GHTECH	1.01394.068	Density gradient centrifugation solution (pH adjustment)
Optima™ XPN-100	Beckman Coulter	A94469	Ultracentrifugation for BEVs isolation at Step 4, 7
OptiPrep™	Serumwerk Bernburg AG	1893	Density gradient centrifugation stock solution
Orbital Shaker	Youning	CS-100	Dissolve feces at Step 2
Phosphate buffered saline	Procell	PB180327	Dissolve feces at Step 2
Pipettor	Eppendorf	3120000267, 3120000259	Transfer the solution
Plastic pasteur pipette	ABCbio	ABC217003-4	Remove supernatant in preprocessing at Step 3.4
Polyvinylidene difluoride (PVDF) membranes	Millipore	ISEQ00010, IPVH00010	Used in western blotting for protein transfer at Step 8.5.5
Prefabricated polyacrylamide gel, 4–20% 15 Wells	ACE	F15420Gel	Used in western blotting for protein separation at Step 8.5.2, 8.5.3
Primary antibody diluent	Fdbio science	FD0040	Used in western blotting at Step 8.5.8
Protein ladder	Fdbio science	FD0672	Used in western blotting and Coomassie brilliant blue stain at Step 8.5
Rapid protein blotting solution	UBIO	UW0500	Used in western blotting for protein transfer at Step 8.5.5
Rotor SW 32 Ti Swinging-Bucket Rotor	Beckman Coulter	369650	Ultracentrifugation for BEVs isolation at Step 4, 7
Syringe 20 mL, 50 mL	Jetway	ZSQ-20ML, YCXWJZSQ-50 mL	Transfer buffers and remove supernatant in preprocessing
TBS powder	Fdbio science	FD1021	Used in western blotting at Step 8.5
Transmission electron microscope (TEM)	Hitachi	H-7650	Morphological observation for BEVs at Step 8.3
Tween-20	Fdbio science	FD0020	Used in western blotting at Step 8.5
Ultracentrifuge tube	Beckman	326823, 355642	Ultracentrifugation for BEVs isolation at Step 4, 7
Ultra-clean bench	AIRTECH	SW-CJ-2FD	Perform the procedures about liquid handling
Water bath	Bluepard	CU600	Used for measuring protein content of BEVs at Step 8.2.5
ZetaView	Particle Metrix	S/N 21-734, Software ZetaView (version 8.05.14 SP7)	Nanoparticle tracking analysis (NTA) for measuring the particle size and concentration of BEVs at Step 8.4