

# Scalable Isolation and Purification of Extracellular Vesicles from *Escherichia coli* and Other Bacteria

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## Materials

Name	Company	Catalog Number	Comments
0.5 mL flat cap, thin-walled PCR tubes	Thermo Scientific	3430	it is important to use thin-walled PCR tubes to obtain accurate readings with Qubit
16% Paraformaldehyde (formaldehyde) aqueous solution	Electron microscopy sciences	15700	
250 mL Fiberlite polypropylene centrifuge bottles	ThermoFisher	010-1495	
500 mL Fiberlite polypropylene centrifuge bottles	ThermoFisher	010-1493	
65 mm Polypropylene Round-Bottom/Conical Bottle Adapter	Beckman Coulter	392077	Allows Vivacell to fit in rotor
<i>Akkermansia mucinophila</i>	ATCC	BAA-835	
Amicon-15 (100 kDa MWCO)	MilliporeSigma	UFC910024	
Avanti J-20 XPI centrifuge	Beckman Coulter		No longer sold by Beckman. Avanti J-26XP is closest contemporary model.
<i>Bacteroides thetaiotaomicron</i> VPI 5482	ATCC	29148	
<i>Bifidobacterium breve</i>	NCIMB	B8807	
<i>Bifidobacterium dentium</i>	ATCC	27678	
Brain Heart infusion (BHI) broth	Himedia	M2101	After autoclaving, Both BHI broth and agar were introduced into the anaerobic chamber, supplemented with Menadione (1 µg/L), hematin (1.2 µg/L), and L-Cysteine Hydrochloride (0.05%). They were then incubated for at least 24 h under anaerobic conditions before inoculation with the anaerobic bacterial strains.
C-300 microfluidics cartridge	Spectradyne		
Chloramphenicol	MP Biomedicals	ICN19032105	

Electron microscope	FEI company	Tecnai G2 SpiritBT	
<i>Escherichia coli</i> HST08 (Stellar competent cells)	Takara	636763	
<i>Escherichia coli</i> MP1	Dr. Mark Goulian (gift)		commensal bacteria derived from mouse gut
Fiberlite 500 mL to 250 mL adapter	ThermoFisher	010-0151-05	used with Fiberlite rotor to enable 250 mL bottles to be used for smaller size of starting bacterial culture
Fiberlite fixed-angle centrifuge rotor	ThermoFisher	F12-6x500-LEX	fits 6 x 500 mL bottles
Formvar Carbon Film 400 Mesh, Copper	Electron microscopy sciences	FCF-400-CU	
Glutaraldehyde (EM-grade, 10% aqueous solution)	Electron microscopy sciences	16100	
Hematin	ChemCruz	207729B	Stock solution was made in 0.2 M L-histidine solution as 1.2 mg/mL
Infinite M Nano+ Microplate reader	Tecan		This equipment was used to measure the mCherry fluorescence
In-Fusion HD Cloning Plus	Takara	638909	For cloning of the PCR fragments into the PCR-linearized vectors
JS-5.3 AllSpin Swinging-Bucket Rotor	Beckman Coulter	368690	
Lauria Bertani (LB) broth, Miller	Difco	244620	
L-Cysteine Hydrochloride	J.T. Baker	2071-05	It should be weighed and added directly to the autoclaved BHI media inside the anaerobic chamber
Masterflex Fitting, Polypropylene, Straight, Female Luer to Hose Barb Adapter, 1/8" ID; 25/PK	cole-parmer - special	HV-30800-08	connection adapters for filtration tubing circuit
Masterflex Fitting, Polypropylene, Straight, Male Luer to Hose Barb Adapter, 1/8" ID; 25/PK	cole-parmer - special	HV-30800-24	connection adapters for filtration tubing circuit
Masterflex L/S Analog Variable-Speed Console Drive, 20 to 600 rpm	Masterflex	HV-07555-00	
Masterflex L/S Easy-Load Head for Precision Tubing, 4-Roller, PARA Housing, SS Rotor	Masterflex	EW-07514-10	
Masterflex L/S Precision Pump Tubing, PharmaPure, L/S 16; 25 ft	Cole Palmer	EW-06435-16	low-binding/low-leaching tubing
Menadione (Vitamin K3)	MP	102259	Stock solution was made in ethanol as 1 mg/mL
MIDIKROS 41.5CM 100K MPES 0.5MM FLL X FLL 1/PK	Repligen	D04-E100-05-N	TFF device we have used to filter up to 2 L of <i>E. coli</i> culture supernatant
Nano-Glo Luciferase Assay System	Promega	N1110	This assay kit was used to measure the luminescence of the nluc reporter protein
NanoLuc (Nluc) Luciferase Antibody, clone 965808	R&D Systems	MAB10026	
nCS1 microfluidics resistive pulse sensing instrument	Spectradyne		
nCS1 Viewer	Spectradyne		Analysis software for particle size distribution
OneTaq 2x Master Mix with Standard Buffer	NEB	M0482	DNA polymerase master mix used to perform the routine PCR reactions for colony checking
Protein LoBind, 2.0 mL, PCR clean tubes	Eppendorf	30108450	

Q5 High-Fidelity 2x Master Mix	NEB	M0492	DNA polymerase master mix used to perform the PCR reactions needed for cloning
qEV original, 35 nm	Izon		maximal loading volume of 0.5 mL
qEV rack	Izon		for use with the qEV-original SEC columns
qEV-2, 35 nm	Izon		maximal loading volume of 2 mL
Qubit fluorometer	ThermoFisher		Item no longer available. Closest available product is Qubit 4.0 Fluorometer (cat. No. Q33238)
Qubit protein assay kit	ThermoFisher	Q33211	Store kit at room temperature. Standards are stored at 4 °C.
Sorvall Lynx 4000 centrifuge	ThermoFisher	75006580	
SpectraMax i3x Microplate reader	Molecular Devices		This equipment was used to measure the nanoluciferase bioluminescence
Stericup Quick-release-GP Sterile Vacuum Filtration system (150, 250, or 500 mL)	MilliporeSigma	S2GPU01RE S2GPU02RE S2GPU05RE	One or multiple filters can be used to accommodate working volumes. In our experience, you can filter twice the volume listed on the product size.
Uranyl acetate	Electron microscopy sciences	22400	
Vinyl anaerobic chamber	Coy Lab		
Vivacell 100, 100,000 MWCO PES	Sartorius	VC1042	
Whatman Anotop 10 Plus syringe filters (0.02 micron)	MilliporeSigma	WHA68093002	to filter MRPS diluent