

Isolation and Characterization of Cyanobacterial Extracellular Vesicles

Steven J. Biller^{*1}, María del Carmen Muñoz-Marín^{*2}, Steeve Lima^{3,4,5}, Jorge Matinha-Cardoso^{3,4}, Paula Tamagnini^{3,4,6}, Paulo Oliveira^{3,4,6}

¹Department of Biological Sciences, Wellesley College ²Departamento de Bioquímica y Biología Molecular, Campus de Excelencia Internacional, Agroalimentario, Universidad de Córdoba ³i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto ⁴IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto ⁵MCbiology Doctoral Program, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto ⁶Departamento de Biologia, Faculdade de Ciências, Universidade do Porto

* These authors contributed equally

Corresponding Authors

Steven J. Biller
sbiller@wellesley.edu

María del Carmen Muñoz-Marín
Marín
b32mumam@uco.es

Paulo Oliveira
paulo.oliveira@ibmc.up.pt

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Materials

Name	Company	Catalog Number	Comments
1x Phosphate buffered saline (PBS), pH 7.4	Home-made buffer	---	Standard wash/storage buffer which can be used with vesicles; can be made in lab or purchased commercially
2% Uranyl acetate solution	Electron Microscopy Sciences	22400-2	Negative staining - TEM
4x Laemmli Sample Buffer	Bio-Rad	161-0747	Denaturation of vesicle sample prior to proteolysis, required for lipopolysaccharides (LPS) staining
Amicon Ultra Centrifugal Filters (100 kDa)	Merck	UFC9100XX	Alternative option for centrifugal ultrafiltration
BG11 medium	Home-made medium	---	Medium for cultivation of <i>Synechocystis</i> sp. PCC 6803
Carbon Support Film 200 Mesh, copper. CF200-Cu	Electron Microscopy Sciences	71150	Transmission electron microscopy (TEM) grid
easiGlow Glow Discharge Cleaning System	Ted Pella	91000	Commercial TEM grid glow discharger
EMbed 812 epoxy resin	Electron Microscopy Sciences	14120	Ultra-thin sections - TEM
Filter holder for vacuum system	Thermo Fisher Scientific	300-4000	Reusable units with filter membrane support plates
Glutaraldehyde Grade I	Sigma-Aldrich	G5882-10X1ml	Ultra-thin sections - TEM
HEPES [N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) sodium salt]	Sigma-Aldrich	H7006	Buffering agent for cyanobacterial growth media
Lead Citrate, Trihydrate	Electron Microscopy Sciences	17800	Stain for use in electron microscopy
Macrosep Advance Centrifugal Devices with Omega Membrane 100K	Pall	MAP100C36	For centrifugal ultrafiltration of small volume samples

Millipore Pellicon 3 TFF Module	EMD Millipore	XX42P0060 or XX42P0080	Alternative TFF option for concentrating large volume samples
Milli-Q Reference Water Purification System	Merck	Z00QSV0WW	Water purification system for obtaining type I ultrapure grade water
NanoSight	Malvern Panalytical	LM14 or NS300	Nanoparticle tracking analysis
Optima L80 XP Ultracentrifuge	Beckman Coulter	L80 XP	Ultracentrifuge (or similar model)
OptiPrep / Iodixanol	Sigma-Aldrich	D1556	Density gradient media
Osmium Tetroxide Reagent Plus	Sigma-Aldrich	201030	Ultra-thin sections - TEM
Pall Centramate PE TFF holder	Pall Corporation	FS002K10	TFF module good for concentrating 10s-100s of L of sample; requires additional 30 or 100 kDa filter modules to scale with your estimated volumes
Peristaltic pump Masterflex L/S	Cole-Parmer	07559-07	Pump for driving large-scale TFF modules
Piston Gradient Fractionator	Biocomp Instruments	152-001	Automated density gradient fractionation
Polycarbonate tubes for the 70 Ti rotor	Beckman Coulter	355618	Reusable ultracentrifuge polycarbonate aluminum tubes with cap assembly
Pro99 medium	Home-made medium	---	Medium for cultivation of <i>Prochlorococcus</i>
Pro-Q Emerald LPS Gel Stain Kit	Thermo Fisher Scientific	P20495	LPS staining
SN medium	Home-made medium	---	Medium for cultivation of cyanobacterial marine strains such as <i>Synechococcus</i>
Sodium cacodylate trihydrate	Sigma-Aldrich	C0250-100G	Buffering agent in the preparation of vesicles samples for TEM
SW32Ti swinging bucket rotor	Beckman Coulter	369650	Ultracentrifuge rotor, holds 6x ~40 mL tubes; good for pelleting of bulk material
SW60Ti swinging bucket rotor	Beckman Coulter	335650	Ultracentrifuge rotor, holds 6x ~4.5 mL tubes; good for gradient purifications and final vesicle washes
Syringe 1mL Luer	BD Plastipak	303172	For vesicle isolation and loading samples into nanoparticle tracking analysis (NTA) equipment
Syringe filter 0.2 µm	Pall Corporation	4602	For vesicle isolation from cultures
TAPS [N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid]	Sigma-Aldrich	T5130	Buffering agent for cyanobacterial growth media
Transmission electron microscope	JEOL	JEM-1400	Or similar microscope
Type 70 Ti Fixed-Angle Titanium Rotor	Beckman Coulter	337922	Ultracentrifuge rotor, holds 8x ~39 mL tubes; good for pelleting of bulk material
Type XIV protease from <i>Streptomyces griseus</i>	Sigma-Aldrich	P5147	Enzyme for proteolysis of EVs proteins, required for LPS staining
UltraClear tubes for the SW32Ti rotor	Beckman Coulter	344058	Single use ultracentrifuge tubes
UltraClear tubes for the SW60Ti rotor	Beckman Coulter	344062	Single use ultracentrifuge tubes
Ultramicrotome PowerTome XL, PT-PC	RMC Products, Boeckeler Instruments	75501	Microtome for ultra-thin sections - TEM
Universal 320 R centrifuge	Hettich	Z654736	This or any similar general-purpose benchtop/floor standing centrifuge can be used for pelleting cells
Vacuum apparatus	KNF Neuberger	N026.3 AT.18	Or any similar vacuum pump and trap

Vivaflow 200 100,000 MWCO PES	Sartorius	VF20P4	TFF module, good for 2-3L. You can connect different modules for higher volume (figure 1).
Whatman Polycap TC Capsule filter (0.2/0.2µm)	Cytiva	6717-9502	Capsule filter for filtering large volumes of liquid
Zetasizer	Malvern Panalytical	Zetasizer Nano ZS	Dynamic light scattering (DLS) instrument