

Materials List for:

Directed Protein Packaging within Outer Membrane Vesicles from *Escherichia coli*: Design, Production and Purification

Nathan J. Alves^{1,2}, Kendrick B. Turner¹, Scott A. Walper¹

¹Center for Bio/Molecular Science & Engineering, Naval Research Laboratory

²Department of Emergency Medicine, Indiana University of School of Medicine

Correspondence to: Scott A. Walper at Scott.Walper@nrl.navy.mil

URL: <https://www.jove.com/video/54458>

DOI: [doi:10.3791/54458](https://doi.org/10.3791/54458)

Materials

Name	Company	Catalog Number	Comments
IPTG	Any		Always prepare fresh or aliquot and freeze.
L-arabinose	Any		Can be prepared ahead of time and stored at 4 °C.
Ampicillin	Any		Add immediately prior to use after media cools sufficiently from being autoclaved.
Chloramphenicol	Any		Add immediately prior to use after media cools sufficiently from being autoclaved.
TB/LB Culture Media	Any		Other growth medias will likely work similarly.
Triton X-100	Any		One of many potential suitable surfactants.
Baffled culture flasks	Any		The baffles promote higher levels of aeration.
CHES	Fisher Bioreagents	BP318-100	Optimal buffer used for paraoxon degradation (pH> 8).
Paraoxon	Chem Service	N-12816	Very toxic substance to be handled carefully and disposed of properly.
Syringe Filter 0.45 µm	Thermo Scientific	60183-221 (30 mm)	Filter diameter will depend on volume of sample. Low protein binding membrane is critical.
Shaker incubator	New Brunswick	Excella E24	Precise temperature and mixing is essential for reproducible bacterial growth.
Sorvall Culture Centrifuge	Thermo Scientific	RC 5B PLUS	Large volume (500 ml) culture centrifuge capable of 7,000 x g.
Sorvall Ultracentrifuge	Thermo Scientific	WX Ultra 90	Capable of centrifugal forces ≥150,000 x g.
Ultracentrifuge Rotor	Thermo Scientific	AH-629	Ensure the proper rotor and tubes are used and that everything is properly balanced.
Ultra-Clear Ultracentrifuge Tubes (25 x 89 mm)	Beckman Coulter	344058	Ensure no stress fractures are present prior to use and that tubes are precisely balanced.
Spectrophotometer	Tecan	Infinite M1000	Necessary for enzyme kinetic assays.
DLS/particle tracking	NanoSight	LM10	Necessary for OMV size distribution and concentration determination.

BL21(DE3)	NEB		Suitable bacterial expression strain.
pET22	EMD Millipore	69744-3	Other plasmids can be used in place of these.
pACYC184	NEB		Other plasmids can be used in place of these.
Gel Extraction Kit	Qiagen	28704	Example kit.