

A Simple Benchtop Filtration Method to Isolate Small Extracellular Vesicles from Human Mesenchymal Stem Cells

Benson Koh^{1,2}, Kian Leong Tan³, Hong Hao Chan⁴, Qi Hao Daniel Looi², Moon Nian Lim⁵, Chee Wun How⁴, Jia Xian Law¹, Jhi Biau Foo^{6,7}

¹Centre for Tissue Engineering and Regenerative Medicine, Universiti Kebangsaan Malaysia Medical Centre ²My CytoHealth Sdn. Bhd. ³School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University ⁴School of Pharmacy, Monash University Malaysia ⁵Haematology Unit, Cancer Research Centre, Institute for Medical Research (IMR), National Institutes of Health (NIH), Ministry of Health Malaysia ⁶School of Pharmacy, Faculty of Health & Medical Sciences, Taylor's University ⁷Centre for Drug Discovery and Molecular Pharmacology (CDDMP), Faculty of Health and Medical Sciences, Taylor's University

Corresponding Author

Jhi Biau Foo

JhiBiau.Foo@taylors.edu.my

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Materials

Name	Company	Catalog Number	Comments
40% acrylamide	Nacalai Tesque	06121-95	Western blot
95% ethanol	Nacalai Tesque	14710-25	Disinfectants
Absolute Methanol	Chemiz	45081	To activate PVDF membrane (Western blot)
Accutase	STEMCELL Technologies	7920	Cell dissociation enzyme
ammonium persulfate	Chemiz	14475	catalyse the gel polymerisation (Gel electrophoresis)
anti-CD 81 (B-11)	Santa Cruz Biotechnology	sc-166029	Antibody for sEVs marker
anti-TSG 101 (C-2)	Santa Cruz Biotechnology	sc-7964	Antibody for sEVs marker
Bovine serum albumin	Nacalai Tesque	00653-31	PVDF membrane blocking
bromophenol blue	Nacalai Tesque	05808-61	electrophoretic color marker
Centricon Plus-70 (100 kDa NMWL)	Millipore	UFC710008	sEVs isolation
ChemiLumi One L	Nacalai Tesque	7880	chemiluminescence detection reagent
CryoStor Freezing Media	Sigma-Aldrich	C3124-100ML	Cell cryopreserve
Dulbecco's modified Eagle's medium	Nacalai Tesque	08458-45	Cell culture media
ExcelBand Enhanced 3-color High Range Protein Marker	SMOBIO	PM2610	Protein molecular weight markers
Extra thick blotting paper	ATTO		buffer reservoir (Western blot)
Glycerol	Merck	G5516	Chemicals for western blot
Glycine	1st Base	BIO-2085-500g	Chemicals for buffer (Western Blot)
horseradish peroxidase-conjugated mouse IgG kappa binding protein (m-IgGkBP-HRP)	Santa Cruz Biotechnology	sc-516102	Secondary antibody (Western Blot)

Human Wharton's Jelly derived Mesenchymal Stem Cells (MSCs)	Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, The National University Malaysia		
mouse antibodies anti-CD 9 (C-4)	Santa Cruz Biotechnology	sc-13118	Antibody for sEVs marker
Nanosight NS300 equipped with a CMOS camera, a 20 × objective lens, a blue laser module (488 nm), and NTA software v3.2	Malvern Panalytical, UK		
paraformaldehyde	Nacalai Tesque	02890-45	Sample Fixation during TEM
penicillin–streptomycin	Nacalai Tesque	26253-84	Antibiotic for media
phenylmethylsulfonyl fluoride	Nacalai Tesque	27327-81	Inhibit proteases in the sEVs samples after adding lysis buffer
phosphate-buffered saline	Gibco	10010023	Washing, sample dilution
polyvinylidene fluoride (PVDF) membrane with 0.45 mm pore size	ATTO		To hold protein during protein transfer (Western blot)
protease inhibitor cocktail	Nacalai Tesque	25955-11	Inhibit proteases in the sEVs samples after adding lysis buffer
Protein Assay Bicinchoninate Kit	Nacalai Tesque	06385-00	Protein measurement
sample buffer solution with 2-ME	Nacalai Tesque	30566-22	Reducing agent for western blot
sodium chloride	Nacalai Tesque	15266-64	Chemicals for western blot
sodium dodecyl sulfate	Nacalai Tesque	31606-62	ionic surfactant during gel electrophoresis
Tecnaï G2 F20 S-TWIN transmission electron microscope	FEI, USA		
tetramethylethylenediamine	Nacalai Tesque	33401-72	chemicals to prepare gel
tris-base	1st Base	BIO-1400-500g	Chemicals for buffer (Western Blot)
Tween 20	GeneTex	GTX30962	Chemicals for western blot
UVP (Ultra Vision Product) CCD imager			CCD imager for western blot signal detection