

Materials List for:

Preparation of Exosomes for siRNA Delivery to Cancer Cells

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Materials

Name	Company	Catalog Number	Comments
Material			
Sterile Newborn Calf Serum Heat Inactivated	First Link	08-05-850	500 ml
EMEM medium	Thermo Fisher Scientific	11090081	500 ml
Penicillin/Streptomycin	Thermo Fisher Scientific	15140-122	100 ml
GlutaMax	Thermo Fisher Scientific	35050-038	100 ml
Sucrose	Fisher Scientific	S/8600/60	1 kg
Deuterium oxide (D ₂ O)	Sigma-Aldrich	151882	250 g
1X Phosphate Buffered Saline	Thermo Fisher Scientific	10010015	500 ml
Glycine	VWR Chemicals	101196X	1 kg
Sepharose CL-2B	GE Healthcare Life Sciences	17-0140-01	1 L; Particle Size 60 µm-200 µm
Trypsin-EDTA 0.05%	Thermo Fisher Scientific	25300096	100 ml
Aldehyde/sulfate latex beads	Thermo Fisher Scientific	A37304	4% w/v, 4 µm, 15 ml
MicroBCA kit	Thermo Fisher Scientific	23235	
CD81 antibody (APC)	Thermo Fisher Scientific	17-0819-42	Lot: E15950-104. RRID: AB_11150235. 1:10 dilution in 50 µl sample
CD81 isotype (APC)	Thermo Fisher Scientific	17-4714-81	Lot: 4291563. RRID: AB_763650. 1:10 dilution in 50 µl sample
CD9 antibody (FITC)	Thermo Fisher Scientific	11-0098-41	Lot: 4345870. RRID: AB_10698007. 1:10 dilution in 50 µl sample
CD9 isotype (FITC)	Thermo Fisher Scientific	11-4714-41	Lot: 4299784. RRID: AB_10598647. 1:10 dilution in 50 µl sample
CD63 antibody (PE)	Thermo Fisher Scientific	12-0639-41	Lot: 1930435. RRID: AB_2572564. 1:10 dilution in 50 µl sample
CD63 isotype (PE)	Thermo Fisher Scientific	12-4714-81	Lot: 1937696. RRID: AB_470059. 1:10 dilution in 50 µl sample
Atto655-siRNA	Eurogentec	SQ-SIRNA	(Labelled-S) UGC-GCU-ACG-AUC-GAC-GAU-G55; (Unlabelled-AS) CAU-CGU-CGA-UCG-UAG-CGC-A55.
Equipment			
Milllex-GP Syringe Filter Unit 0.22 µm	Millipore	SLGP033RS	
CELLine AD1000 bioreactor flasks	Wheaton	WCL1000ad	
Ultracentrifuge	Beckman Coulter	Optima XPN-80	

Swing-out rotor	Beckman Coulter	SW45 Ti	
Fixed-angle rotor	Beckman Coulter	Type 70 Ti	
Ultracentrifuge tubes	Beckman Coulter	355631	Polycarbonate. Max. fill 32 ml
Ultracentrifuge bottles	Beckman Coulter	355618	Polycarbonate. Min. fill 16 ml, max. fill 25 ml
NanoSight	Malvern	LM10	Software: NanoSight NTA v3.2
Flow Cytometer	BD Biosciences	FACSCalibur	
Centrifuge	Eppendorf	5810R	
Plate reader	BMG Labtech	FLUOstar Omega	
Flow cytometry tubes	BD Biosciences, Falcon	352052	
Microfuge tubes	Starlab	S1615-5500	1.5 ml
Cell culture flasks	Fisher Scientific	156499	75 cm ²
Transmission electron microscope	FEI Electron Optics	Philips CM 12	with Tungsten filament and a Veleta - 2k × 2k side-mounted TEM CCD Camera (Olympus, Japan)
Amaxa Nucleofector I	Lonza	Nucleofector I	with Amaxa's Nucleofector Kits
24-well flat-bottom plates	Corning	Costar	
Blunt fill needle	BD Biosciences	305180	18G x 1 1/2" (1.2 mm x 40 mm)
Glass pipettes	Fisher Scientific	1156-6963	
Name	Company	Catalog Number	Comments
Reagents			Composition
Normal medium			Eagle's Minimum Essential Medium supplemented with 10% foetal bovine serum (FBS), 1% penicillin/streptomycin and 1% GlutaMax.
Exosome-depleted medium			Eagle's Minimum Essential Medium supplemented with 10% exosome-depleted FBS (see below), 1% penicillin/streptomycin and 1% GlutaMax.
Exosome-depleted FBS			Subject FBS to ultracentrifugation at 100,000 g for 18 h at 4°C. The FBS supernatant post-centrifugation was collected and sterile-filtered using 0.22 µm filters.
25% w/w sucrose cushion			Add 1.9 g (± 0.001 g) of sucrose in a universal tube, and top up with D ₂ O until the weight reaches 7.6 g (± 0.001 g). This makes ~6ml of the 25% w/w sucrose cushion.
3% FBS/PBS			Add 1.5 ml exosome-depleted FBS to 48.5 ml 1X PBS to prepare 50 ml of 3% FBS/PBS.
100 µM BSA solution			Prepare 1mM BSA solution by adding 0.3325 g to 50 ml PBS. Make 1:10 dilution of this stock to obtain 100 µM BSA solution. Make 5-10 µl aliquots of this 100 µM BSA solution (for single use) and store them at -20°C. All BSA solution should be stored at -20°C, and discard solutions that have undergone ≥2 freeze-thaw cycles.

100 mM glycine solution			Add 0.375 g of glycine to 50 ml PBS to obtain 100 mM glycine solution, store at 4°C.
Citric acid buffer with EDTA			Mix 0.1954 g citric acid and 0.2087 g disodium phosphate in 50 mL of deionised water. Add EDTA to 0.1 mM. Adjust pH to 4.4.
Fixing solution			Prepare formaldehyde/ glutaraldehyde, 2.5% (w/v) each in 0.1 M sodium cacodylate buffer, and adjust pH to 7.4.
Aqueous uranyl acetate			Prepare 25% (v/v) uranyl acetate in methanol.
50% methanol/H2O			Mix methanol and H2O 1:1 (v/v).
SEC Column packed with Sepharose CL-2B			Dilute 37.5 ml Sepharose CL-2B resin with 12.5 ml PBS to obtain 75% Sepharose CL-2B solution. Pour the Sepharose CL-2B solution into a column of dimension 2.9 cm [H] x 1.3 cm [W]. Pour the Sepharose CL-2B to 3-4 mm above the stated height, and then press it down to the stated height with the filter for optimal packing.