

Materials List for:

Cell Culture on Silicon Nitride Membranes and Cryopreparation for Synchrotron X-ray Fluorescence Nano-analysis

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Materials

Name	Company	Catalog Number	Comments
Ammonium Acetate solution, BioUltra, for molecular biology, ~5M in H ₂ O	SIGMA	09691-250mL	One can prepare the required solution from high-grade ammonium acetate powder and ultrapure water, pH and osmolarity needs to be adjusted anyway.
B27 supplement, 50x	Life Technologies, Invitrogen	17504-044	for hippocampal neuron culture
Dulbecco's Phosphate Buffered Saline, DPBS, ([-] CaCl ₂ , [-] MgCl ₂)	GIBCO	14190-094	cell culture
DMEM with Phenol Red/Glutamax I (Medium ATCC modification)	GIBCO	21885025	cell culture
Dulbecco's modified Eagle medium (DMEM)	Life Technologies, Invitrogen	31966-02	for hippocampal neuron culture
Dumont Tweezers #5, Straight Self-closing, 0.05x0.01mm Tips, Biology	World Precision Instrument	501202	
Emitech K750X Peltier-Cooled EM Freeze Dryer	Quorum Technology	EK3147	
Ethane N45	Air Liquid	p0505s05r0a001	C ₂ H ₆ > 99,995 %
Fetal Bovine Serum, Performance Plus, certified One Shot format, US origin	GIBCO	A31604-02	cell culture
HBSS 10x	Life Technologies, Invitrogen	14185-052	for hippocampal neuron culture
Leica GP quick-release forceps	Leica	16706435	
MDA-MB-231 cell line, an epithelial, human adenocarcinoma breast cancer cell	ATCC	ATCC HTB-26	cell culture
Neurobasal medium	Life Technologies, Invitrogen	21103-049	for hippocampal neuron culture
Nunc 4-Well Plate	Thermo Fisher	176740	cell culture
Osmo1 Single-Sample Micro-Osmometer	Advanced Instruments	Osmo1	Alternative can be found at Fisher scientific (Wescor Inc. VAPRO® Vapor Pressure Osmometer)
Penicillin-Streptomycin	SIGMA	P4333	cell culture
poly-L-lysine	SIGMA	P4707	Other type of coating can be used that is dependent of the cell type to be cultured on the membrane, other adhesion factors such as fibronectin, collagen, polyornithine can be tested accordingly. Cell can be cultured directly on silicon nitride membrane, but the latter are slightly hydrophobic and adhesion

			factors are recommended unless the membrane are processed to be hydrophilic (glow plasma discharged).
Plunge freezing robot Leica EM GP main unit	Leica	16706401	Alternative for automated plunger are the Vitrobot Mark IV (FEI), CryoPlunge 3 (Gatan), MS-002 Rapid Immersion Freezer (EMS). Manual home-made system can be used but an environment-controlled chamber is an asset for plunge-freezing.
Silicon nitride membrane (Si3N4)	Silson Ltd.	SiRN-5.0(o)-200-1.5-500-NoHCl	The proposed silicon nitride membrane type is optimised for analysis at ID16A ESRF X-ray nanoprobe, The 500 nm thickness of the membrane was chosen being more robust for cellular manipulation and cryofixation detailed within this protocol. Membrane with thickness of 200 nm or below can also be used although quite fragile, and other design of silicon nitride membrane can be purchased (for example TEM compatible membrane...) from Sison or other company such as Norcada, SPI supplies, Ted Pella, EMS, LabTech, Neyco...
Trypan blue solution 0.4%	GIBCO	15250061	cell culture
Trypsin-EDTA, 0.05%	GIBCO	25300-054	cell culture
Ultrapure Elemental Analysis Grade, Ultrapure Water	Fisher Chemicals	W9-1	MilliQ water can be used but has to be tested for trace element level of contamination using for example ICP-MS analysis.
Whatman No. 1 filter paper with precut hole	Leica	16706440	Alternative filter paper may be used and must have an outer diameter of 55 mm, the Punch for filter paper system from Leica (ref.16706443) can be used.