

Materials List for:

Expression and Purification of the Human Lipid-sensitive Cation Channel TRPC3 for Structural Determination by Single-particle Cryo-electron Microscopy

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Materials

Name	Company	Catalog Number	Comments
pEG BacMam vector (pFastBacI)	addgene	31488	
DH10α cells	Life Technologies	10361-012	
S.O.C. media	Corning	46003CR	for transformation of DH10α cells for Bacmid
Bacmam culture plates	Teknova	L5919	for culture of transformed DH10α cells
Incubation shaker for bacterial cells	Infors HT	Multitron standard	
Incubated orbital shaker for insect cells	Thermo-Fisher	SHKE8000	
Reach-in CO ₂ incubator for mammalian cells	Thermo-Fisher	3951	
Table-top orbital shaker	Thermo-Fisher	SHKE416HP	used in Reach-in CO ₂ incubator for mammalian cells
Incubator	VWR	1535	for bacterial plates
QIAprep Spin Miniprep Kit	Qiagen	27106	for plasmid extraction and purification
Phenol:Chloroform:Isoamyl alcohol	Invitrogen	15593031	for DNA extraction
Sf9 cells	Life Technologies	12659017	insect cells for producing virus
Sf-900 media	Gibco	12658-027	insect cell media
FBS	Atlanta Biologicals	S11550	
Cellfectin II	Gibco	10362100	for transfecting insect cells
lipofectamine 2000	Invitrogen	11668-027	for transfecting mamalian cells
0.2 mm syringe filter	VWR	28145-501	for filtering P1 virus
0.2 mm filter flasks 500ml resevoir	Corning	430758	for filtering P2 virus
erlenmeyer culture flask (flat bottom 2L)	Gene Mate	F-5909-2000	for culturing insect cells
erlenmeyer culture flask (baffled 2L)	Gene Mate	F-5909-2000B	for culturing mammalian cells
nanodrop 2000 spectrophotometer	Thermo-Fisher	ND-2000	for determining DNA and protein concentrations
HEK293	ATCC	CRL-3022	mammalian cells for producing protein
Freestyle 293 expression Medium	Gibco	1238-018	mammalian cell media for protein expression

Butyric Acid Sodium Salt	Acros	263195000	to amplify protein expression
PMSF	Acros	215740500	protease inhibitor
Aprotinin from bovine lung	Sigma-Aldrich	A1153-100MG	protease inhibitor
Leupeptin hydrochloride	Sigma-Aldrich	24125-16-4	protease inhibitor
pepstatin A	Fisher Scientific	BP2671-250	protease inhibitor
digitonin	EMD Millipore	300410	detergent - to solubilize protein from membrane
imidazole	Sigma	792527	to elute protein from resin column
TALON resin	Clontech	635504	for affinity purification by His-tag
superose6 incese columns	GE	29091596; 29091597	for HPLC and FPLC
Prominence Modular HPLC System	Shimadzu	See Below	
Controller Module	"	CBM20A	
Solvent Delivery System	"	LC30AD	
Fluorescence Detector	"	RF20AXS	
Autosampler with Cooling	"	SIL20ACHT	
Pure FPLC System with Fractionator	Akta		
thrombin (alpha)	Haematologic Technologies Incorporated	HCT-0020 Human alpha	for cleaving GFP tag
Amicon Ultra 15 mL 100K centrifugal filter tube	Millipore	UFC910008	for concentrating protein
EDTA	Fisher	E478500	for stabilizing protein
400 mesh carbon-coated copper grids	Ted Pella Inc.	01754-F	grids for negative stain
Quantifoil holey carbon grid (gold, 1.2/1.3 µm size/hole space, 300 mesh)	Electron Microscopy Sciences	Q3100AR1.3	grids for Cryo-EM
Vitrobot Mark III	FEI		for preparing sample grids by liquid ethane freezing
liquid nitrogen	Dura-Cyl	UN1977	
ethane gas	Airgas	UN1035	
Solarus Plasma System	Gatan	Model 950	for cleaning grids before sample freezing
Tecnai Spirit electron microscope	FEI		for negative stain EM imaging
Talos Arctica electron microscopce	FEI		for screening and low resolution imaging of Cryo-EM grids
Titan Krios electron microscope	FEI		for high-resolution Cryo-EM imaging
Software			
Gautomatch software	http://www.mrc-lmb.cam.ac.uk/kzhang/Gautomatch/		to pick particles from micrographs
Relion 2.1 software	https://github.com/3dem/relion		to construct 2D and 3D classification
CryoSPARC software	https://cryosparc.com/		to generate an initial structure model
Frealign software	http://grigoriefflab.janelia.org/frealign		to refine particles
Coot software	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/cool/		to build a model
MolProbity software	http://molprobity.biochem.duke.edu/		to evaluate the geometries of the atomic model

SerialEM software	http://bio3d.colorado.edu/SerialEM/		for automated serial image stack acquisition
MotionCor2 software	http://msg.ucsf.edu/em/software/motioncor2.html		for motion correction of summed movie stacks
GCTF software	https://www.mrc-lmb.cam.ac.uk/kzhang/Gctf/		for measuring defocus values in movie stacks
Phenix.real_space_refine software	https://www.phenix-online.org/documentation/reference/real_space_refine.html		for real space refinement of the initial 3D model