

Fluorescence-Based Measurements of Phosphatidylserine/Phosphatidylinositol 4-Phosphate Exchange Between Membranes

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Materials

Name	Company	Catalog Number	Comments
L-cysteine ≥97 % (FG)	Sigma	W326305-100G	Prepare a 10 mM L-cysteine stock solution in water. Aliquots are stored at -20 °C
2 mL Amber Vial, PTFE/Rub Lnr, for lipids storage in CHCl ₃	Wheaton	W224681	
4 mm-diameter glass beads	Sigma	Z265934-1EA	
50 mL conical centrifuge tube	Falcon		
ÄKTA purifier	GE healthcare		FPLC
Aluminium foil			
Amicon Ultra-15 with a MWCO of 3 and 10 kDa	Merck	UFC900324, UFC901024	
Amicon Ultra-4 with a MWCO of 3 and 10 kDa	Merck	UFC800324, UFC801024	
Ampicillin			Prepare a 50 mg/mL stock solution with filtered and sterilized water and store it at -20 °C.
Bestatin	Sigma	B8385-10mg	
BL21 Gold Competent Cells	Agilent		
C16:0 Liss (Rhod-PE) in CHCl ₃ (1 mg/mL)	Avanti Polar Lipids	810158C-5MG	
C16:0/C16:0-PI(4)P	Echelon Lipids	P-4016-3	Dissolve 1 mg of C16:0/C16:0-PI(4)P powder in 250 µL of MeOH and 250 µL of CHCl ₃ . Then complete with CHCl ₃ to 1 mL. The solution must become clear.
C16:0/C18:1-PS (POPS) in CHCl ₃ (10 mg/mL)	Avanti Polar Lipids	840034C-25mg	
C18:1/C18:1-PC (DOPC) in CHCl ₃ (25 mg/mL)	Avanti Polar Lipids	850375C-500mg	

CaCl ₂	Sigma		Prepare 10 mM CaCl ₂ stock solution in water.
Cell Disruptor	Constant Dynamics		
Chloroform (CHCl ₃) RPE-ISO	Carlo Erba	438601	
Complete EDTA-free protease inhibitor cocktail	Roche	5056489001	
Deionized (Milli-Q) water			
Dimethylformamide (DMF), anhydrous, >99% pure			
DNAse I Recombinant, RNAse free, in powder	Roche	10104159001	
DTT	Euromedex	EU0006-B	Prepare 1 M DTT stock solution in Milli-Q water. Prepare 1 mL aliquots and store them at -20 °C.
Econo-Pac chromatography columns (1.5 × 12 cm).	Biorad	7321010	
Electroporation cuvette 2 mm	Ozyme	EP102	
Electroporator Eppendorf 2510	Eppendorf		
Fixed-Angle Rotor Ti45 and Ti45 tubes	Beckman	Spinning the bacterial lysates	
Glass-syringes (10, 25, and 50 µL) for fluorescence experiment	Hamilton		
Glass-syringes (25 , 100, 250, 500, and 1000 µL) to handle lipid stock solutions	Hamilton	1702RNR, 1710RNR, 1725RNR, 1750RN type3, 1001RN	
Glutathione Sepharose 4B beads	GE Healthcare	17-0756-05	
Glycerol (99% pure)	Sigma	G5516-500ML	
Hemolysis tubes with a cap			
HEPES ,>99 % pure	Sigma	H3375-500G	
Illustra NAP 10 desalting column	GE healthcare	GE17-0854-02	
Isopropyl β-D-1-thiogalactopyranoside (IPTG)	Euromedex	EU0008-B	Prepare 1 M IPTG stock solution in Milli-Qwater. Prepare 1 mL aliquots and store them at -20 °C.
K-Acetate	Prolabo	26664.293	
Lennox LB Broth medium without glucose			Prepared with milli-Q water and autoclaved.
Liquid nitrogen	Linde		
Methanol (MeOH) ≥99.8%	VWR	20847.24	
MgCl ₂	Sigma		Prepare a 2 M MgCl ₂ solution. Filter the solution using a 0.45 µm filter.
Microplate 96 Well PS F-Botom Black Non-Binding	Greiner Bio-one	655900	
Mini-Extruder with two 1 mL gas-tight Hamilton syringes	Avanti Polar Lipids	610023	
Monochromator-based fluorescence plate reader	TECAN	M1000 Pro	
N,N'-Dimethyl-N-(Iodoacetyl)-N'-(7-Nitrobenz-2-Oxa-1,3-Diazol-4-yl)Ethylendiamine (IANBD Amide)	Molecular Probes		Dissolve 25 mg of IANBD in 2.5 mL of dimethylsulfoxide (DMSO) and prepare 25 aliquot of 100 µL in 1.5 mL screw-cap tubes. Do not completely screw the cap. Then, remove DMSO in a freeze-dryer to obtain 1 mg of dry IANBD per tube. Tubes are closed and stored at -20 °C in the dark.
NaCl	Sigma	S3014-1KG	

PBS			137 mM NaCl, 2.7 mM KCl, 10 mM NaH ₂ PO ₄ , 1.8 mM KH ₂ PO ₄ , autoclaved and stored at 4 °C.
Pear-shaped glass flasks (25 mL, 14/23, Duran glass)	Duran Group		
Pepstatin	Sigma	p5318-25mg	
pGEX-C2 _{LACT} plasmid			Available on request from our lab
pGEX-PHF _{APP} plasmid			Available on request from our lab
Phenylmethylsulfonyl fluoride (PMSF) ≥98.5% (GC)	Sigma	P7626-25g	Prepare a 200 mM PMSF stock solution in isopropanol
Phosphoramidon	Sigma	R7385-10mg	
Polycarbonate filters (19 mm in diameter) with pore size of 0.2 µm	Avanti Polar Lipids	610006	
Poly-Prep chromatography column (with a 0-2 mL bed volume and a 10 mL reservoir)	Biorad	7311550	
Prefilters (10 mm in diameter).	Avanti Polar Lipids	610014	
PyMOL	http://pymol.org/		Construction of the 3D models of the proteins (Figure 1A)
Quartz cuvette for UV/visible fluorescence (minimum volume of 600 µL)	Hellma		
Quartz cuvettes	Hellma		
Refrigerated centrifuge Eppendorf 5427R	Eppendorf		
Rotary evaporator	Buchi	B-100	
Screw-cap microcentrifuge tubes (1.5 mL)	Sarsted		
Small magnetic PTFE stirring bar (5 × 2 mm)			
Snap-cap microcentrifuge tubes (0.5, 1, and 2 mL)	Eppendorf		
SYPRO orange			fluorescent stain to detect protein in SDS-PAGE gel
Thermomixer	Starlab		
THROMBIN, FROM HUMAN PLASMA	Sigma	10602400001	Dissolve 20 units in 1 mL of milli-Q water and prepare 25 µL aliquots in 0.5 mL Eppendorf tubes. Then freeze and store at -80 °C.
Tris, ultra pure	MP	819623	
Ultracentrifuge L90K	Beckman		
UV/Visible absorbance spectrophotometer	SAFAS		
UV/visible spectrofluorometer with a temperature-controlled cell holder and stirring device	Jasco or Shimadzu	Jasco FP-8300 or Shimadzu RF-5301PC	
Vacuum chamber			
Water bath	Julabo		
XK 16/70 column packed with Sephacryl S200HR	GE healthcare		