

Materials List for:

Dynamic Proteomic and MiRNA Analysis of Polysomes from Isolated Mouse Heart After Langendorff Perfusion

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Materials

Name	Company	Catalog Number	Comments
Pentobarbital	Vortech Pharmaceuticals	9373	for euthansia
Heparin	Sagent	103424	used in langendorff preparation
forceps	Fine Science Tools	91110-10	used to hang the heart
Langendorff system	Radnoti + home made	n/a	A 'four heart' system consisting of custom blown glass, tubing and water baths
NaCl	Sigma	S7653-5KG	Krebs buffer and Sucrose gradient
KCl	Sigma	P5405	Krebs buffer and Lysis buffer
KH ₂ PO ₄	Sigma	P-5504	Krebs buffer
MgSO ₄	Sigma	M7774-500G	Krebs buffer
Glucose	Sigma	G5767	Krebs buffer
CaCl ₂	Sigma	C1016-500G	Krebs buffer
Sucrose powder	Sigma	S0389-1KG	Sucrose gradient
MgCl ₂	Sigma	208337	Sucrose gradient and Lysis buffer
Tris-base	Sigma	T1503-1KG	Sucrose gradient and Lysis buffer
Xylene Cyanole	Sigma	X-4126	Sucrose gradient
Cycloheximide	Sigma-aldrich	239763	Sucrose gradient and Lysis buffer
RNaseOUT	Life Technologies	C00019	RNAse inhibitor for Lysis buffer
Igepal CA-360 (NP40)	Sigma	I3021	Lysis buffer
Protease Inhibitor Cocktail tablets, EDTA free	Roche	5056489001	
Tube, Thinwall, Ultra-Clear, 13.2 mL, 14 mm x 89 mm	Beckman Coulter	344059	
Ultracentrifuge	Beckman	LE-80K	Ultracentrifugation of the gradients
Rotor	Beckman	SW41	Ultracentrifugation of the gradients
Biologic LP (pump)	Biorad	731-8300	Fractionation of the gradients
BioFrac	Biorad	741-0002	Fractionation of the gradients
Eppendorf RNA/DNA LoBind microcentrifuge tubes, 2 mL tube	Sigma	Z666513-100EA	Gradient fraction and RNA extraction
TRIzol Reagent	Life technologies	AM9738	RNA extraction
Luciferase Control RNA	Promega	L4561	RNA extraction
Chloroform	Fisher Scientific	C606-4	RNA extraction
Glycogen, RNA grade	Thermo Fisher Scientific	R0551	RNA extraction
Isopropanol	Sigma	I9516	RNA extraction

Ethanol	Sigma	E7023-1L	RNA extraction
iScript cDNA Synthesis Kit	BioRad	170-8891	Reverse transcription
iTaq Universal SYBR Green Supermix	BioRad	175-5122	Quantative PCR
miRNeasy Micro Kit (50)	Qiagen	217084	Kit for total RNA isolation
miScript II RT Kit (50)	Qiagen	218161	Kit for miRNA reverse transcription
miScript Sybr Green PCR Kit (200)	Qiagen	218073	Kit for real-time PCR expression analysis of miRNAs
Centrifuge 5424R	Eppendorf		For centrifugation of 1.5 mL or 2.0 mL tubes at different temperatures. Max speed - 21130 x g
Centrifuge 5810R	Eppendorf		For real-time PCR plate centrifugation at different temperatures. Max speed - 2039 x g
My Cycler Thermal Cycler	Bio-Rad		For reverse transcription
CFX96 Real-Time System/C1000 Touch Thermal Cycler	Bio-Rad		For real-time PCR analysis
miRNeasy Serum/Plasma Spike-in Control	Qiagen	219610	For quality control of RNA isolation
Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, green/clear	Bio-Rad	HSP9641	For real-time PCR analysis
Microseal 'B' PCR Plate Sealing Film, adhesive, optical	Bio-Rad	MSB1001	For real-time PCR plate sealing
Research plus Single-Channel Pipette, Gray; 0.5-10 µL	Eppendorf	UX-24505-02	For pipetting
PIPETMAN Classic Pipets, P20	Gilson	F123600G	For pipetting
PIPETMAN Classic Pipets, P200	Gilson	F144565	For pipetting
Rainin L-1000XLS Pipet-Lite XLS LTS Pipette 100-1000 µL	Gilson	17011782	For pipetting
Glycogen, RNA grade	Thermo Fisher Scientific	R0551	Improves total RNA isolation efficiency
Posi-Click 1.7 mL Tubes, natural color	Denville	C2170	RNA isolation and storage; reagent mix
Thermal Cycling Tubes -0.2 mL Individual Caps, Standard 0.2 mL tubes with optically	Denville	C18098-4 (1000910)	Reverse transcription reaction
Sharp 10 Precision barrier Tips	Denville	P1096-FR	For pipetting
Sharp 20 Precision barrier Tips	Denville	P1121	For pipetting
Sharp 200 Precision barrier Tips	Denville	P1122	For pipetting
Tips LTS 1 mL Filter	Rainin	RT-L1000F	For pipetting
miScript Primer Assay (200)	Qiagen	(it changes according to the miRNA)	For real-time PCR analysis
Gradient Master ver 5.3 Model 108	BioComp Instruments		For preparation of sucrose gradients
trichloroacetic acid	Sigma Aldrich	T6399	
acetone	Sigma Aldrich	650501	
Tris hydrochloride	Amresco	M108	
dithiothreitol	Fisher Scientific	BP172	
iodoacetamide	Gbiosciences	RC-150	
sequencing grade modified trypsin, porcine	Promega	V5111	

ammonium bicarbonate	BDH	BDH9206	
formic acid, Optima LC/MS	Fisher Chemical	A117	
methanol, Optima LC/MS	Fisher Chemical	A454	
acetonitrile, Optima LC/MS	Fisher Chemical	A996	
Protein LoBind tubes 0.5 mL	Eppendorf AG	22431064	
Protein LoBind tubes 1.5 mL	Eppendorf AG	22431081	
HLB μ Elution plate 30 μ m	Oasis	186001828BA	
SpeedVac concentrator	Thermo Scientific	Savant SPD2010	
sonicator	Qsonica	Oasis180	
centrifuge	Thermo Scientific	Sorvall Legend micro 21R	
LC trap column PepMap 100 C18	Thermo Scientific	160454	
LC separation column PepMap RSLC C18	Thermo Scientific	164536	
mass spectrometer	Thermo Scientific	Orbitrap Elite ion trap mass spectrometer	
MSConvert software	ProteoWizard Toolkit		
Sorcerer-SEQUEST software	Sage-N Research, Inc.		