

Materials List for:

# The Lactate Dehydrogenase Sequestration Assay — A Simple and Reliable Method to Determine Bulk Autophagic Sequestration Activity in Mammalian Cells

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## Materials

Name	Company	Catalog Number	Comments
1.5 ml and 2 ml microcentrifuge tubes	Eppendorf	211-2130 and 211-2120	
12-well plates	Falcon	353043	
Accumax cell detachment solution	Innovative Cell Technologies	A7089	Keep aliquots at -20 °C for years, and in fridge for a few months
Bafilomycin A1	Enzo	BML-CM110-0100	Dissolve in DMSO
BJ cells	ATCC	CRL-2522	use at passage <30
Bovine serum albumin (BSA)	VWR	422361V	
Burker counting chamber	Fisher Scientific	139-658585	
Countess Cell Counting Chamber Slides	ThermoFisher Scientific	C10228	
Countess II Automated Cell Counter	ThermoFisher Scientific	AMQAX1000	
Cover glass for the Burker counting chamber	Fisher Scientific	139-658586	
Criterion Tris-HCl Gel, 4–20%, 26-well, 15 µl, 13.3 x 8.7 cm (W x L)	Bio-Rad	3450034	
DTT	Sigma-Aldrich	D0632	
Earle's balanced salt solution (EBSS)	Gibco	24010-043	contains 0.1% glucose
EDTA	Sigma-Aldrich	E7889	
Electroporation cuvette (4 mm)	Bio-Rad	1652088	
Exponential decay wave electroporator	BTX Harvard Apparatus	EMC 630	
Fetal bovine serum (FBS)	Sigma	F7524	10% final concentration in RPMI 1640 medium
HEK293 cells	ATCC	CRL-1573	
Imidazole	Sigma-Aldrich	56750	Autoclave a 65 mM solution and keep in fridge for months
Incubator; Autoflow IR Direct Heat CO2 incubator	NuAire	NU-5510E	
Lipofectamine RNAiMAX Transfection Reagent	ThermoFisher	13778150	
LNCaP cells	ATCC	CRL-1740	use at passage <30
3-Methyl Adenine (3MA)	Sigma-Aldrich	M9281	Stock 100 mM in RPMI in -20 °C. Heat stock to 65 °C for 10

			minutes, and use at 10 mM final concentration
Refridgerated Microcentrifuge	Beckman Coulter Life Sciences	368831	
Refridgerated Microcentrifuge with soft-mode function	Eppendorf	Eppendorf 5417R	
MRT67307 hydrochloride (ULKi)	Sigma-Aldrich	SML0702	Inhibits ULK kinase activity. Dissolve in DMSO.
MaxMat Multianalyzer instrument	Erba Diagnostics	PL-II	
MCF7 cells	ATCC	HTB-22	
NADH	Merck-Millipore	1.24644.001	
Nycodenz	Axis-Shield	1002424	
Opti-MEM Reduced Serum Medium	ThermoFisher	31985062	
Phosphate-buffered saline (PBS)	Gibco	20012-019	
Pipette tips 3 (0.5-20 µl)	VWR	732-2223	Thermo Fischer ART Barrier tips
Pipette tips (1-200 µl)	VWR	732-2207	Thermo Fischer ART Barrier tips
Pipette tips (100-1000µl)	VWR	732-2355	Thermo Fischer ART Barrier tips
Pipettes	ThermoFisher	4701070	Finnpipette F2 GLP Kit
Poly-D-lysine	Sigma-Aldrich	P6407-10X5MG	Make a 1 mg/ml stock solution in sterile H <sub>2</sub> O. This solution is stable at -20 °C for at least 1 year.
Pyruvate	Merck-Millipore	1066190050	
RPE-1 cells (hTERT RPE-1)	ATCC	CRL-4000	
RPMI 1640	Gibco	21875-037	
SAR-405	ApexBio	A8883	Inhibits phosphoinositide 3-kinase class III (PIK3C3). Dissolve in DMSO.
Silencer Select Negative Control #1 (siCtrl)	ThermoFisher/Ambion	4390843	
Silencer Select ATG9-targeting siRNA (siATG9A)	ThermoFisher/Ambion	s35504	
Silencer Select FIP200-targeting siRNA (siFIP200)	ThermoFisher/Ambion	s18995	
Silencer Select ULK1-targeting siRNA (siULK1)	ThermoFisher/Ambion	s15964	
Silencer Select ULK2-targeting siRNA (siULK2)	ThermoFisher/Ambion	s18705	
Silencer Select GABARAP-targeting siRNA (siGABARAP)	ThermoFisher/Ambion	s22362	
Silencer Select GABARAPL1-targeting siRNA (siGABARAPL1)	ThermoFisher/Ambion	s24333	
Silencer Select GABARAPL2-targeting siRNA (siGABARAPL2)	ThermoFisher/Ambion	s22387	
Sodium phosphate monobasic dihydrate (NaH <sub>2</sub> PO <sub>4</sub> • 2H <sub>2</sub> O)	Merck-Millipore	1.06580.1000	
Sodium phosphate dibasic dihydrate (Na <sub>2</sub> HPO <sub>4</sub> • 2H <sub>2</sub> O )	Prolabo	28014.291	
Sucrose	VWR	443816T	10% final concentration in water; filter through 0.45 µm filter and keep in fridge for months
Thapsigargin	Sigma-Aldrich	T9033	Inhibits the SERCA ER Ca <sup>2+</sup> pump. Dissolve in DMSO.
Triton X-405	Sigma-Aldrich	X405	1% final

Trypan Blue stain 0.4%	Molecular Probes	T10282	
Trypsin-EDTA (0.25% w/v Trypsin)	Gibco	25200-056	
Tween-20	Sigma-Aldrich	P2287	0.01% final