

Materials List for

Imaging ATG9A, a Multi-Spanning Membrane Protein

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jove.com/video/65349

Materials

Name	Company	Catalog Number	Comments
24 multiwell plates	Falcon	353047	For tissue culture
35 mm Dish No. 1.5 Coverslip 14 mm Glass Diameter Uncoated	MATTEK	P35G-1.5-14-C	Cell culture dish for live-cell microscopy
4x Laemmli Sample Buffer	Bio-Rad	1610747	
60 mm tissue culture dish	Thermofisher Scientific	10099170	For tissue culture
Alexa Fluor 647 Phalloidin	Thermofisher Scientific	A22287	Actin stain
anti-ATG9A antibody	home made	STO-215	Rabbit anti N-terminal peptide ATG9A
anti-Rabbit IgG, peroxidase-linked	Invitrogen	10794347/NA934-1ml	Secondary antibody for rabbit polyclonal STO-215
anti-RFP antibody	Evrogen	AB233	for Western Blot
ATG9A Monoclonal Antibody (14F2 8B1), Invitrogen	Invitrogen	15232826	Antibody for immunofluorescence
ATG9A-eGFP	home made		Construct which expresses tagged ATG9A
Bemis Parafilm	Thermofisher Scientific	11747487	self-sealing thermoplastic film
Bio-Rad Protein Assay Dye Reagent Concentrate	Bio-Rad	5000006	For determining protein concentration
Bovine serum albumin (BSA)	Merck	10735086001	For blocking non-specific labelling
CaCl ₂ ·2H ₂ O	/		For PBS and EBSS
cOmplete Protease Inhibitor Cocktail	Roche	11697498001	Supplement in lysis buffer to prevent protein degradation
Cy3 AffiniPure Goat Anti-Armenian Hamster IgG	Jackson ImmunoResearch	127-165-099	Secondary antibody for i14F2 8B1 antibody for immunofluorescence
Cyclohexamide	Sigma Aldrich	66-81-9	To stop protein translation
D-Glucose	/		For EBSS
Digitonin	Merck	300410	For permeabilizing cells
DMEM	Merck	D6546-6x500ml	For tissue culture
eGFP-ATG9A	home made		Construct which expresses tagged ATG9A
Fetal Bovine Serum	Gibco	10270-106	Supplement for DMEM for cell culture

FIJI (ImageJ)	/	https://fiji.sc/	Open source image analysis software
Hoechst	Thermofisher Scientific	H3570	Stains the nucleus
KCl	/		For EBSS
L-glutamine	Sigma	67513	For tissue culture
Lipofectamine 2000	Invitrogen	11668-019	For Cell Transfection
LSM880 Airyscan microscope	Zeiss	/	Confocal microscopy
MgCl ₂	/		For PBS
MgSO ₄ .7H ₂ O	/		For EBSS
Mowiol mounting solution	Millipore	475904	for permanent mounting glass coverslips
NaCl	/		For EBSS
NaH ₂ PO ₄ .2H ₂ O	/		For EBSS
NaHCO ₃	/		For EBSS
NuPAGE 3 to 8%, Tris-Acetate, 1.5 mm, Mini Protein Gels	Thermofisher Scientific	EA0378BOX	for Western Blotting
NuPAGE MES SDS Running Buffer (20x)	Life Tech	NP0002	for Western Blotting
Opti-MEM I Reduced Serum Medium	Thermofisher Scientific	31985062	For Cell Transfection
Paraformaldehyde	Agar Scientific	R1026	For fixing cells
pcDNA3.1-mCherry-3xFlag-ATG9A	home made		Construct which expresses tagged ATG9A
Phosphate Buffered Saline (PBS)	/		For tissue culture
PNGaseF	NEB	P0710S	To remove N-linked glycans
Poly-D-lysine hydrobromide mol wt 70,000-150,000	Merck	P0899	For coating coverslips
Rapid PNGase F enzyme	NEB	P07105	To remove N-linked glycans
RFP-ATG9A	home made		Construct which expresses tagged ATG9A
Triton X-100	Thermofisher Scientific	13454259	Detergent for Cell lysis
Trypsin-EDTA solution	Sigma	T4049	For tissue culture
Whatman Filter Paper	Merck	WHA1001325	For Western Blot and IF
XCell SureLock Mini-Cell Electrophoresis System	Invitrogen	EI0001	For Western Blotting
Zen Black edition	Zeiss	/	Used to operate the LSM 880