

Materials List for:

***In Vitro* and *In Vivo* Detection of Mitophagy in Human Cells, *C. Elegans*, and Mice**

Evandro F. Fang^{1,6}, Konstantinos Palikaras², Nuo Sun³, Elayne M. Fivenson¹, Ryan D. Spangler⁴, Jesse S. Kerr¹, Stephanie A. Cordonnier¹, Yujun Hou¹, Eszter Dombi⁵, Henok Kassahun⁶, Nektarios Tavernarakis^{2,7}, Joanna Poulton⁵, Hilde Nilsen⁶, Vilhelm A. Bohr^{1,8}

¹Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health

²Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas

³Center for Molecular Medicine, National Heart Lung and Blood Institute, National Institutes of Health

⁴Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health

⁵Nuffield Department of Obstetrics and Gynaecology, University of Oxford

⁶Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital

⁷Department of Basic Sciences, Faculty of Medicine, University of Crete

⁸Danish Center for Healthy Aging, University of Copenhagen

Correspondence to: Evandro F. Fang at evandrofeifang@yahoo.com; e.f.fang@medisin.uio.no, Vilhelm A. Bohr at BohrV@grc.nia.nih.gov

URL: <https://www.jove.com/video/56301>

DOI: [doi:10.3791/56301](https://doi.org/10.3791/56301)

Materials

Name	Company	Catalog Number	Comments
mt-Keima mouse	Jackson Laboratory		
Lipofectamine 2000 DNA transfection reagent	Thermofisher	#11668027	
Opti-MEM medium (Gibco)	Thermofisher	#31985062	serum-free medium
mtKemia plasmid: pCHAC-mt-mKeima	addgene	#72342	
COXII antibody (mouse)	abcam	#ab110258	
LAMP2 antibody (rabbit)	NOVUS	#CD107b	
goat-anti-rabbit with wavelength 568 nm of red fluorescent protein (RFP)	Thermofisher	#Z25306	Alexa Fluor 568 dye
goat-anti-mouse with wavelength 488 nm of green fluorescent protein (GFP)	Thermofisher	#Z25002	Alexa Fluor 488 dye
prolong gold antifade with DAPI	Invitrogen	#P36931	
6-well plate	SIGMA Corning Costar	#CLS3516	
4-well chamber slide	Thermofisher, Nunc Lab-Tek	#171080	
Nunc F 96-well plate	Thermofisher	#152038	
LC3B antibody rabbit	NOVUS	#NB100-2220	
DNA antibody	Progen Biotechnik	#anti-DNA mouse monoclonal, AC-30-10	
DAPI	Thermofisher	#D1306	antifade mounting medium with DAPI
IN Cell analyzer (fluorescent reader)	GE Healthcare Life Sciences	#IN Cell analyzer 2200	
Eclipse TE-2000e confocal microscope	Nikon	#TE-2000e	
Colocalization software	Volocity	#Volocity 6.3	alternative Zeiss ZEN 2012 software
IN Cell Investigator Software	GE Healthcare Life Sciences	#28408974	

cell culture medium	Thermofisher	#DMEM–Dulbecco's Modified Eagle Medium	
Penicillin-Streptomycin (10,000 U/mL)	Thermofisher	#15140122	
Fetal Bovine Serum	Sigma-Aldrich	#12003C-1000ML	
Cell culture Incubator	Thermofisher	#Thermo Forma 3110 CO2 Water Jacketed Incubator	
epifluorescence microscope	Zeiss		Zeiss Axio Imager Z2
camera	Olympus		Olympus DP71
confocal microscope	Zeiss		Zeiss Axio Observer Z1
confocal software	Zeiss		ZEN 2012
image analysis software	Image J	colocalization analysis, etc	https://imagej.nih.gov/ij/
statistical analysis software	GraphPad Software Inc., San Diego, USA		GraphPad Prism software package
material to make a worm pick	Surepure Chemetals	#4655	The pick is made of 30 gauge 90% platinum 10% iridium wire
IR: N2;Ex[<i>pmyo-3</i> TOMM-20::Rosella]	Material inquiry to Tavernarakis Nektarios		Maintain transgenic animals at 20 °C
IR: N2; Ex[<i>pdct-1</i> DCT-1::GFP; <i>pmyo-3</i> DsRed::LGG-1]	Material inquiry to Tavernarakis Nektarios		
<i>pmyo-3</i> TOMM-20::Rosella	Material inquiry to Tavernarakis Nektarios		
<i>pdct-1</i> DCT-1::GFP	Material inquiry to Tavernarakis Nektarios		
<i>pmyo-3</i> DsRed::LGG-1	Material inquiry to Tavernarakis Nektarios		
Paraquat solution	see supplementary data for preparation		
M9 buffer	see supplementary data for preparation		
M9-levamisole buffer	see supplementary data for preparation		
Glass Microscope Slides and Coverslips	Fisher Scientific	#B9992000	
Surgical forceps	STERIS Animal Health	19 Piece Canine Spay Pack Economy	
Surgical scissors	STERIS Animal Health	19 Piece Canine Spay Pack Economy	
1x PBS	Thermofisher	#AM9625	10x PBS needs to be diluted to 1x PBS by using ddH2O
shaker	Fisher Scientific	#11-676-178	Thermo Scientific MaxQ HP Tabletop Orbital Small Open Air Platform Shaker Package A
2% agarose pad	see supplementary data for preparation		
Vibroslice blades	World precision instruments	#BLADES-2	single-edge blade
metal plate	MSC	#78803988	0.012 in thick x 6 in wide x 12 in long, 430 Stainless Steel Sheet
Triton X-100			detergent
Methyl viologen dichloride hydrate	Sigma-Aldrich	#856177	paraquat
Incubator for nematodes	AQUALYTIC		Incubator to maintain 20 °C
Dissecting stereomicroscope	Olympus	SMZ645	
Confocal microscope	Zeiss	AxioObserver Z1	For nematodes (step 2)

epifluorescence microscope	Zeiss	Axiomager Z2	For nematodes (step 2)
UV crosslinker	Vilber Lourmat	BIO-LINK – BLX-E365	UV light source; 356 nm