

Materials List for:

Assessing Autophagic Flux by Measuring LC3, p62, and LAMP1 Co-localization Using Multispectral Imaging Flow Cytometry

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Materials

Name	Company	Catalog Number	Comments
15 mL centrifuge tube	Falcon	352096	
50 mL centrifuge tube	Falcon	352070	
AF647 labeled Donkey anti-Mouse - IgG	Invitrogen	A-31571	Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 647. Concentration 2 mg/mL. https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Secondary-Antibody-Polyclonal/A-31571
anti-human CD107a-PE (LAMP1)	BioLegend	328607	Clone H4A3. Concentration 400 µg/mL. http://www.biolegend.com/pe-anti-human-cd107a-lamp-1-antibody-4967.html
anti-human CD45- AF488	BioLegend	304017	Clone HI30. http://www.biolegend.com/alexa-fluor-488-anti-human-cd45-antibody-2738.html
anti-human CD45- PE	BioLegend	304008	Clone HI30. http://www.biolegend.com/pe-anti-human-cd45-antibody-708.html
anti-human CD45-AF647	BioLegend	304018	Clone HI30. http://www.biolegend.com/alexa-fluor-647-anti-human-cd45-antibody-2739.html
Anti-LC3 (Human) mAb clone 4E12	MBL International Corporation	M152-3	Clone 4E+12. Concentration 2 mg/mL. https://www.mblintl.com/products/m152-3
Anti-SQSTM1 / p62 antibody [EPR4844] - Autophagosome Marker (Alexa Fluor 488)	abcam	ab185015	Clone EPR4844. Concentration 0.5 mg/mL. http://www.abcam.com/sqstm1-p62-antibody-epr4844-autophagosome-marker-alexa-fluor-488-ab185015.html
Chloroquine diphosphate Salt	Sigma-Aldrich	C6628-25G	
Cleanser - Coulter Clenz	Beckman Coulter	8546931	Fill container with 200 mL of Cleanser. https://www.beckmancoulter.com/wsrportal/page/itemDetails?itemNumber=8546931#2/10/0/25/1/0/asc/2/8546931///0/1/0/
DAPI Stain 5 mg/mL	MilliporeSigma	508741	http://www.emdmillipore.com/US/en/product/DAPI-Stain---CAS-28718-90-3---Calbiochem,EMD_BIO-508741

Debubbler - 70% Isopropanol	MilliporeSigma	1.3704	Fill container with 200 mL of Debubbler. http://www.emdmillipore.com/US/en/product/2-Propanol-70%25-%28V%2FV%29-0.1-%C2%B5m-filtred,MDA_CHEM-137040?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
Dulbecco's Phosphate Buffered Saline 10x	MilliporeSigma	BSS-2010-B	Ca++Mg++ Free
Dulbecco's Phosphate Buffered Saline 1x	MilliporeSigma	BSS-1006-B	PBS Ca++Mg++ Free
Earle's Balanced Salt Solution 1x	Gibco	14155	
Fetal Bovine Serum	HyClone	SH30071.03	
Formaldehyde, 10%, methanol free, Ultra Pure	Polysciences, Inc.	04018	This is what is used for the 4% and 1% Formalin. CAUTION: Formalin/Formaldehyde toxic by inhalation and if swallowed. Irritating to the eyes, respiratory systems and skin. May cause sensitization by inhalation or skin contact. Risk of serious damage to eyes. Potential cancer hazard. http://www.polysciences.com/default/catalog-products/life-sciences/histology-microscopy/fixatives/formaldehydes/formaldehyde-10-methanol-free-pure/
Hanks' Balanced Salt Solution 1x	Gibco	14175	
Jurkat, Clone E6-1 (ATCC TIB-152)	ATCC	TIB-152	https://www.atcc.org/products/all/TIB-152.aspx
MEM Non-Essential Amino Acids 100x	HyClone	SH30238.01	
MIFC - ImageStreamX Mark II	MilliporeSigma	100220	A 2 camera ImageStreamX Mark II eqiped with the 405nm, 488nm, and 642nm lasers was used. http://www.emdmillipore.com/US/en/life-science-research/cell-analysis/amnis-imaging-flow-cytometers/imagestreamx-Mark-ii-imaging-flow-cytometer/VaSb.qB.QokAAAFzRop.zHe.nav?cid=BI-XX-BDS-P-GOOG-FLOW-B325-0006
MIFC analysis software - IDEAS 6.2	MilliporeSigma	100220	The companion software to the MIFC (ImageStreamX MKII). IDEAS version 6.2
MIFC software - INSPIRE	MilliporeSigma	100220	This is the software that runs the MIFC (ImageStreamX MKII). INSPIRE version 200.1.388.0
PE anti-human CD107a (LAMP-1) Antibody clone H4A3	BioLegend	328608	http://www.biolegend.com/pe-anti-human-cd107a-lamp-1-antibody-4967.html
Penicillin/Streptomycin/Glutamine solution 100x	HyClone	SV30082.1	
Rinse - Ultrapure water or deionized water	NA	NA	You can use any ultrapure water or deionized water. Fill container with 900 mL of Rinse.
RPMI-1640 Medium 1x	HyClone	SH30027.01	
Sheath - PBS	MilliporeSigma	BSS-1006-B	This is the same as Dulbecco's Phosphate Buffered Saline 1X Ca

			++MG++ free. Fill container with 900 mL of Sheath.
Siliconized polypropylene microcentrifuge tubes	Fisherbrand	02-681-320	Fisherbrand Siliconized Low-Retention Microcentrifuge Tubes 1.5 mL. https://www.fishersci.com/shop/products/fisherbrand-siliconized-low-retention-microcentrifuge-tubes-8/p-193936
Sodium Pyruvate solution (100 mM)	HyClone	SH30239.01	
Sterilizer - 0.4 - 0.7% Hypochlorite	VWR	JT9416-1	This is essentially 10% Clorox bleach that can be made by diluting Clorox bleach with water. Fill container with 200 mL of Sterilizer.
System Calibration Reagent - SpeedBead	MilliporeSigma	400041	Each tube holds ~10 mL. https://www.emdmillipore.com/US/en/life-science-research/cell-analysis/amnis-imaging-flow-cytometers/support-training/XDqb.qB.wQMAAFLBDUp.zHu,nav
T75 flask	Falcon	353136	
TRITON X-100, PROTEIN GRADE Detergent, 10% Solution, Sterile-Filtered	MilliporeSigma	648463-50ML	http://www.emdmillipore.com/US/en/product/TRITON-X-100,-PROTEIN-GRADE-Detergent,-10%25-Solution,-Sterile-Filtered---CAS-9002-93-1---Calbiochem,EMD_BIO-648463
Water, Cell Culture Grade	HyClone	SH30529.03	