

Flux-Based Assay for the Identification of Autophagy Modulators for Osteoarthritis

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jove.com/video/61078

Materials

Name	Company	Catalog Number	Comments
100 mm cell culture plate	Corning	430167	Cell culture plate
12-well multiplate	Corning	353043	Cell culture plate
15 mL Centrifuge conical tube	Falcon-Corning	352095	Centrifuge conical tube
24-well multiplate	Corning	351147	Cell culture plate
25 cm ² Cell Culture Flask	Falcon-Corning	353014	Cell culture flask
384-well multiplate Cell Carrier	Perkin Elmer	6007550	Cell culture plate
6-well multiplate	Corning	351146	Cell culture plate
96-well multiplate	Corning	353077	Cell culture plate
Acoustic liquid handling technology	Labcyte	–	https://www.labcyte.com
Chloroquine	Sigma-Aldrich	C6628	autophagic flux inhibitor
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich, St. Louis, MO	D2650	Disolvent
Dulbecco's Modified Eagle's Medium (DMEM)	Lonza, Basel, Switzerland	BE-604F	T/C28a2 growth medium
Eagle's Minimum Essential Medium (EMEM)	ATCC	30–2003	HEK 293-T17 growth medium
FACScalibur cytometer	Becton Dickinson, CA	–	https://www.bdbiosciences.com/en-eu
Fetal Bovine Serum (FBS)	Sigma-Aldrich, St. Louis, MO	F9665	HEK 293-T17 serum
Fetal Calf Serum (FCS)	Gibco by Life Technologies, CA	26010–074	Serum
FuGene	Promega	E2691	A nonliposomal mixture of lipids as a plasmid delivery method to create autophagy reported cell line
Handheld electronic 384 channel pipette	Integra	–	https://www.integra-biosciences.com/united-states/en/electronic-pipettes/viaflo-96384#downloads
Hank's Balanced Salt Solution (HBSS)	Sigma-Aldrich	H6648	Buffer
HEK 293-T17	ATCC	CRL-11268	Kidney cell line. Cells were used to facilitate retroviral packaging

High Content Screening System	Perkin Elmer	-	https://www.perkinelmer.com/es/product/operetta-cls-system-hh16000000
Hoechst 33342	Thermo Fisher Scientific	62249	DNA staining
Image Analysis Software	Perkin Elmer	-	https://www.perkinelmer.com/es/product/harmony-4-9-office-license-hh17000010
Liquid Handler workstation	Perkin Elmer	-	https://www.perkinelmer.com/es/category/janus-liquid-handler-workstations
Microplate washer robot	Biotek	-	https://go.biotek.com/405tradein
Opti-MEM® (1x)	Thermo Fisher Scientific	11058	Transfection medium
Paraformaldehyde (PFA)	Sigma-Aldrich	158127	Fixer
pBABE-puro mCherry-EGFP-LC3B	Addgene, Cambridge, MA	22418	Plasmid
pCL-Eco	Addgene, Cambridge, MA	12371	Plasmid
Penicillin-Streptomycin (P/S)	Sigma-Aldrich	P0781	Antibiotic
Phosphate-buffered saline (PBS)	MP Biomedicals	2810305	Buffer
Puromycin	Sigma-Aldrich, St. Louis, MO	P8833	Antibiotic
Rapamycin	Calbiochem, Germany	5053210	autophagic flux activator
Software CellQuestPro	Becton Dickinson	-	https://www.bdbiosciences.com/en-eu
Syringe filters 0.45 µm	Corning	CLS431220	Sterile filter
T/C28a2	-	-	human chondrocytes cell line
Trypsin	Gibco by Life Technologies, CA	15400054	Trypsin used with T/C28a2 cells
Trypsin	Sigma-Aldrich, St. Louis, MO	SM-2002-C	Trypsin used with HEK 293-T17 cells
VSV.G	Addgene, Cambridge, MA	14888	Plasmid