

Characterization of Neuronal Lysosome Interactome with Proximity Labeling Proteomics

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Materials

Name	Company	Catalog Number	Comments
10% (w/v) Saponin solution	Acros Organics	419231000	Flourescent Microscopy
Accutase	Life Technologies	A1110501	cell detachment solution, Cell Culture
B27 Supplement	Fisher Scientific	17504044	Cell Culture, Cortical Neuron Medium
BDNF	PeptoTech	450-02	Cell Culture, Cortical Neuron Medium
Boric acid	Sigma-Aldrich	B6768	Cell Culture, Borate Buffer
Bovine Serum Albumin	Millipore Sigma	A8806	To make standard solutions to measure total protein concentrations
Brainphys neuronal medium	STEMCELL Technologies	5790	Cell Culture, Cortical Neuron Medium
CD45R (B220) Antibody Alexa Fluor 561	Thermo Fisher Scientific	505-0452-82	Flourescent Microscopy
Chroman1 ROCK inhibitor	Tocris	716310	Cell Culture
cOComplete mini Protease Inhibitor	Roche	4693123001	cocktail inhibitor in Lysis Buffer
DC Protein Assay Kit II	Bio-Rad	5000112	To determine total protein concentrations of cell lysate
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418	Proximity-labeling Reaction
DMEM/F12 medium	Thermo Fisher Scientific	11320082	Cell Culture, Dish Coating
DMEM/F12 medium with HEPES	Thermo Fisher Scientific	11330057	Cell Culture, Induction Medium
Donkey serum	Sigma-Aldrich	D9663	Flourescent Microscopy
Doxycycline hyclate, ≥98% (HPLC)	Sigma-Aldrich	D9891-1G	Cell Culture, Induction Medium
Essential 8 Medium	Thermo Fisher Scientific	A1517001	Cell Culture
Essential 8 Supplement (50x)	Thermo Fisher Scientific	A1517101	Cell Culture
Extraction plate vacuum manifold kit	Waters	WAT097944	For Peptide desalting
Formic Acid (FA)	Fisher Scientific	A11750	For LC-MS analysis
GDNF	PeptoTech	450-10	Cell Culture, Cortical Neuron Medium
Hoechst dye	Thermo Fisher Scientific	62239	Flourescent Microscopy
HPLC grade methanol	Fisher Scientific	A452	For Peptide desalting
HPLC grade water	Fisher Scientific	W5	For Peptide desalting

Human induced pluripotent stem cells	Corriell Institute	GM25256	Cell Culture
Hydrogen peroxide, ACS, 29-32% w/w aq. soln., stab.	Thermo Fisher Scientific	AA33323AD	Proximity-labeling Reaction
Iodoacetamide (IAA)	Millipore Sigma	I6125	For Protein Digestion
Laminin	Fisher Scientific	23017015	Cell Culture, Cortical Neuron Medium
LC-MS grade Acetonitrile	Fisher Scientific	A955	For LC-MS analysis
LC-MS grade water	Fisher Scientific	W64	For LC-MS analysis
L-glutamine	Fisher Scientific	25-030-081	Cell Culture, Induction Medium
Matrigel	Thermo Fisher Scientific	08-774-552	basement membrane matrix, Cell Culture, Dish Coating
Mouse anti-human LAMP1 monoclonal antibody	Developmental Studies Hybridoma Bank	h4a3	Flourescent Microscopy
N-2 Supplement (100x)	Fisher Scientific	17-502-048	Cell Culture, Induction Medium
Nitrocellulose Membrane, Precut, 0.45 µm, 7 x 8.5 cm	Bio-Rad	1620145	To conduct dot blot assay for bead titration
Non-essential amino acids (NEAA)	Fisher Scientific	11-140-050	Cell Culture, Induction Medium
NT-3	PeptoTech	450-03	Cell Culture, Cortical Neuron Medium
Oasis HLB 96-well solid phase extraction plate	Waters	186000309	For Peptide desalting
Odyssey Blocking Buffer (TBS)	LI-COR Biosciences	927-50000	To conduct dot blot assay for bead titration
Paraformaldehyde	Electron Microscopy Sciences	15710	Flourescent Microscopy
Phenol Biotin (1,000x stock)	Adipogen	41994-02-9	Proximity-labeling Reaction
Phosphate-buffered saline (PBS) without calcium or magnesium	Gibco	10010049	Cell Culture, Proximity-labeling Reaction, Flourescent Microscopy
Pierce Quantitative Colorimetric Peptide Assay	Thermo Fisher	23275	Peptide Concentration Assay
Poly-L-Ornithine (PLO)	Millipore Sigma	P3655	Cell Culture, Dish Coating
Sodium Ascorbate	Sigma-Aldrich	A4034	Proximity-Labeling Quench Buffer, Lysis Buffer
Sodium azide	Sigma-Aldrich	S8032	Proximity-Labeling Quench Buffer, Lysis Buffer, Flourescent Microscopy
Sodium chloride	Thermo Fisher Scientific	S271500	Cell Culture, Borate Buffer
Sodium dodecyl sulfate (SDS)	Thermo Fisher Scientific	BP1311220	Lysis Buffer, Dot blot assay buffer, Beads wash buffer
Sodium hydroxide	Sigma-Aldrich	415413	Cell Culture, Borate Buffer
Sodium tetraborate	Sigma-Aldrich	221732	Cell Culture, Borate Buffer
SpeedVac concentrator			vacuum concentrator
Streptavidin Magnetic Sepharose Beads	Cytiva (formal GE)	28-9857-99	Enrich biotinylated proteins
Streptavidin, Alexa Fluor 680 Conjugate	Thermo Fisher Scientific	S32358	To conduct dot blot assay for bead titration
Thermomixer			temperature-controlled mixer
Trifluoacetic acid (TFA)	Millipore Sigma	302031	For Peptide desalting
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)	Millipore Sigma	C4706	For Protein Digestion
Tris-HCl	Thermo Fisher Scientific	BP152500	Lysis Buffer, Dot blot assay buffer, Beads wash buffer
Triton-X	Thermo Fisher Scientific	BP151500	Beads wash buffer
TROLOX	Sigma-Aldrich	648471	Proximity-Labeling Quench Buffer, Lysis Buffer
Trypsin/Lys-C Mix, Mass Spec Grade	Promega	V5073	For Protein Digestion

TWEEN 20	Millipore Sigma	P1379	Dot blot assay buffer
Urea	Thermo Fisher Scientific	BP169500	Beads wash and On-Beads Digestion Buffer
Vitronectin	STEMCELL Technologies	7180	Cell Culture, Dish Coating
Y-27632 ROCK inhibitor	Selleck	S1049	Cell Culture