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# Immunological detection of autophagy and mTORC1related proteins

Forked from Immunological detection of APP and proteins of the endolysosomal system

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## ABSTRACT

Here we present a general protocol for immunological detection by Western blotting MTOR, MTOR (pS2448), ULK1, ULK1 (pS757), p70S6K, p70S6K (pT389), SQSTM1, CALCOCO2, MAP1LC3B, GABARAP, TFEB, TFE3, PGRN, HSP90, and PCNA

DOI

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FORK NOTE



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**KEYWORDS** 

**ASAPCRN** 

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LAST MODIFIED

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PROTOCOL INTEGER ID

68680

MATERIALS TEXT

Α	В	С
REAGENT or	SOURCE	IDENTIFIER
RESOURCE		
Antibodies		
human anti-	R&D Systems	AF2420
progranulin		
anti-PCNA	Santa Cruz Biotechnology	sc56
anti-HSP90	Proteintech	60318-1-lg
anti-TFEB	Cell Signaling Technology	4240
anti-TFE3	Proteintech	14480-1-AP
anti-SQSTM1	Proteintech	18420-1-AP
anti-Calcoco2	Proteintech	12229-1-AP
anti-	Cell Signaling Technologies	2775
MAP1LC3B		
anti-GABARAP	Proteintech	18723-1-AP
anti-MTOR	Cell Signaling Technologies	2983
anti-p70S6K	Cell Signaling Technologies	2708
anti-MTOR	Cell Signaling Technologies	2971
(pS2448)		
anti-ULK1	Cell Signaling Technologies	8054
anti-ULK1	Cell Signaling technologies	14202
(pS757)		
IRDye 680RD	Li-Cor	926-68071
Goat a-Rabbit		
IgG secondary		
antibody		



IRDye 680RD	Li-Cor	926-68070
Goat a-Mouse		
IgG secondary		
antibody		
IRDye 800CW	Li-Cor	926-32211
Goat a-Rabbit		
IgG secondary		
antibody		
IRDye 800CW	Li-Cor	926-32210
Goat a-Mouse		
IgG secondary		
antibody		
Goat a-Rabbit	Cell Signaling Technology	7474P2
IgG, HRP-	och digitaling rediniology	74741 2
linked		
antibody		
Goat a-Rabbit	Bio-Rad	1706515
IgG HRP	Bio-Rau	1700313
_		
conjugate	D: D 1	1706546
Goat a-Mouse	Bio-Rad	1706516
IgG HRP		
conjugate		
Chemicals,		
peptides,		
and		
recombinant		
proteins		
PhosSTOP	Roche	04906845001
Complete	Sigma-Aldrich	11873580001
EDTA-free		
protease		
inhibitor		
cocktail		
REVERT 700	Li-Cor	926-11016
total		320 11010
protein stain		
kit		
NuPAGE LDS	Thermo Fisher	NP0007
sample	Scientific	550/
buffer (4X)	Giontino	
NuPAGE	Thermo Fisher	NP0009
	Scientific	INF UUU 9
sample	SCIENTIFIC	
reducing agent		
(10X)	Die Ded	F000006
Bio-Rad	Bio-Rad	5000006
Protein		
Assay Dye		
Reagent Concentrate		

NuPAGE MES	Thermo Fisher	NP0002
SDS	Scientific	
Running Buffer		
(20X)		
Immobilon-FL	Millipore	IPFL00010
PVDF		
Membrane		
WHEATON	DWK Life Sciences	357542
Dounce		
Tissue Grinder,		
7 mL		
KIMBLE	DWK Life Sciences	885300-0002
KONTES		
Dounce Tissue		
Grinder, 2 mL		7.005
Nonidet P40	Sigma-Aldrich	74385
substitute	Oimman Aldrick	115070
Urea	Sigma-Aldrich	U5378
RIPA lysis and	Thermo Fisher	89900
extraction	Scientific	
buffer		
Experimental		
models: Cell		
lines		
293T cells	ATCC	CRL-3216
293 cells	ATCC	CRL-1573
HeLa cells	ATCC	CCL-2
HeLa cells;	This study and DOI:	
GRN-/-	dx.doi.org/10.17504/protocols.io.4r3l2oxqqv1y/v1	
Software		
and		
algorithms		
ImageLab	Biorad	https://www.bio-rad.com/en-us/product/image-
v6.0.1		lab-software?
		ID=KRE6P5E8Z&source_wt=imagelabsoftware_surl

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## Western blotting

- 1 Lyse cell pellets by homogenization in KPBS buffer, urea buffer, or RIPA buffer with protease and phosphatase inhibitors. FOr some experiments, we employ 293 cells or alternatively HeLa cells with or without the GRN gene, created by CRISPR-based gene editing (DOI: dx.doi.org/10.17504/protocols.io.4r3l2oxqqv1y/v1).
- 2 Determined total protein concentration by BCA or Bradford assay. Normalize samples within a set of samples with additional buffer. Add NuPAGE LDS buffer (4X) plus NuPAGE reducing agent (10X).
- 3 Load samples onto 4-12% NuPAGE Bis-Tris gels (ThermoFisher), and separate by electrophoresis in MES buffer.
- 4 Transfer proteins to PVDF or nitrocellulose membranes by standard wet transfer in 20% methanol.
- 5 Stain membranes with REVERT 700 total protein stain following manufacturer's instructions, and image total protein with a ChemiDoc MP (Bio-Rad) at 680 nm.
- 6 De-stain with REVERT reversal solution for 5 min. Block membranes with tris-buffered saline (TBS) (5% non-fat dry milk) at room temperature for 30-60 min.
- 7 Incubate membranes overnight at 4°C with primary antibody solution in TBS with 0.1% Tween-20 (TBST). Wash six times with TBST for 5 min each. Incubate in secondary antibody solution in TBST (plus 0.01% SDS) for 1h at room temperature.
- 8 Wash membranes four times with TBST for 5 min each.
- 9 When using HRP-conjugated secondary antibodies (Bio-Rad or Cell Signaling Technology), apply luminol and hydrogen peroxide solution to membrane for 2 min, and image membrane with a ChemiDoc MP using the chemiluminescent setting.
- 10 When using Li-Cor fluorescent secondary antibodies, blot membranes dry and image with a ChemiDoc MP at either 800 nm or 680 nm, depending on the secondary antibody.