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Halo-LC3B processing assay to assess autophagy

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details Halo-LC3B processing assay to assess autophagy.

Attachments

738-1854.pdf
115KB

Guidelines

Reference: **DOI**: 10.7554/eLife.78923

Materials

Buffers and reagents:

Growth media: DMEM medium with GlutaMAX containing 10% FBS and 10% Pen-Strep.
1x PBS
Lysis buffer:

	A
	25 mM HEPES pH 7.5
	200 mM NaCl
	2 mM $MgCl_2$
	10% glycerol
	1 mM TCEP
	0.2% n-dodecyl-β-D-maltoside
	pierce protease inhibitors
	Benzonase

DMEM, high glucose, GlutaMAX™ Supplement **Thermo Fisher Catalog #**10566016

Gibco™ Fetal Bovine Serum value heat inactivated (formerly USDA-approved in North America or quali **Fisher Scientific Catalog #**A5256801

Penicillin-Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Scientific Catalog #**15140122

EBSS, calcium, magnesium, phenol red **Thermo Fisher Catalog #**24010043

Janelia Fluor® HaloTag® Ligands **Promega Catalog #**GA1110

Gibco™ Trypsin-EDTA (0.05%) phenol red **Fisher Scientific Catalog #**25-300-120

Pierce Protease Inhibitor Tablets **Thermo Fisher Catalog #**A32963

n-Dodecyl-B-D-maltoside (DDM) **Gold Biotechnology Catalog #** DDM5

Benzonase® Nuclease Purity > 90% **Merck Millipore (EMD Millipore)** **Catalog #**70746-4

NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.0 mm, 12-well **Thermo Fisher** **Catalog #**NP0322BOX

Halo-LC3B processing assay to assess autophagy

1h 50m

1 Generating HeLa cells expressing HaloTag-LC3B using pMRX-IP-HaloTag7-LC3 from Mizushima lab (Addgene #184899; DOI: 10.7554/eLife.78923).

2 Seed HeLa cells at 100-150K cells/well in 12-well plate one day before.

3 Next day, incubate cells with complete DMEM medium and [M] 50 nanomolar (nM) JF646 HaloTag ligand (Promega) for 01:00:00, and then wash twice with 1xPBS. The non-starved samples can be harvested immediately by trypsinization (step 5). 1h

4 To induce autophagy by starvation, treat cells with EBSS buffer (Gibco) for desired period.

5 After the treatment, harvest the cells by trypsinization.

5.1 Wash the wells with 1x PBS.

5.2 Incubate the wells with 0.5 mL trypsin at 37 °C for 00:05:00. 5m

5.3 Add 0.5 mL complete medium into each well.

5.4 Transfer cells into pre-chilled Eppendorf tube, spin 2000 x g, 00:05:00. 5m

5.5 Aspirate off the liquid.

6 Resuspend the cell pellets in 30 µL of lysis buffer and incubate On ice for 00:30:00. 30m

7 Centrifuge the cell lysate at 21000 x g, 00:10:00 . Transfer the cleared lysate into another tube, and measure protein concentration nanodrop spectrophotometer (Thermo Fisher). 10m

8 For each sample, load 20 µg clarified lysates onto NuPAGE 4-12% Bis-Tris Gel (Thermo Fisher).

9 For in-gel fluorescence imaging, the gel was immediately visualized with ChemiDoc MP imaging system (Bio-Rad) after SDS PAGE. Band intensities are acquired by exciting samples at 546 nm (mCherry signal) and 647 nm (JF646 HaloTag ligand signal).