

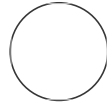


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Halo-LC3B processing assay to assess autophagy

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ABSTRACT

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

ATTACHMENTS

[738-1854.pdf](#)

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

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4qexzv05/v1

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


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83241


Keywords: Halo-LC3B processing assay, autophagy

 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 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 μ L of lysis buffer and incubate  On ice for

30m



 00:30:00


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 μ g 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).