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🌐 Induction of non-selective bulk autophagy

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We use this protocol and it's working

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Abstract

This protocol describes how to induce bulk (non-selective) autophagy in HeLa cells through nutrient starvation.

Attachments



[773-1958.pdf](#)

29KB

Materials

Reagents

- Hank balanced salt medium (HBSS, Thermo Fisher).
- Pierce™ Detergent Compatible Bradford Assay KitThermo FisherCatalog #23246

RIPA buffer

	A	B
	Tris-HCl pH 8.0	50 mM
	NaCl	150 mM
	sodium deoxycholate	0.50%
	SDS	0.10%
	NP-40	1%
	cOmplete mini-EDTA protease inhibitor	
	Roche Phosstop	



Nutrient starvation experiments

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- 1 To induce bulk autophagy, starve the cells by culturing them in Hank balanced salt medium (HBSS, Thermo Fisher) for the indicated time.
- 2 Collect cells by trypsinization, wash them in PBS and lyse them in RIPA buffer for  00:20:00 on ice. Clear the samples by centrifugation at  20000 x g, 4°C, 00:10:00 .
- 3 Collect the soluble supernatant fraction and measure the protein concentrations using the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher).
- 4 Analyse protein samples by SDS-PAGE and western blot analysis.

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