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Co-immunoprecipitation ER-phagy receptors

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

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Abstract

This protocol details the co-immunoprecipitation of ER-phagy receptors.

Materials

pHAGE-TEX264-GFP **addgene Catalog #**201925 or

pHAGE-FAM134C-GFP **addgene Catalog #**201927

Earle's Balanced Salts **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Earle's Balanced Salts

Pierce™ Detergent Compatible Bradford Assay Kit **Thermo Fisher Catalog #**23246

ChromoTek GFP-Trap® Agarose **Proteintech Catalog #** gta-20

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

Lysis buffer

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM
	NP-40	0.5%

Washing buffer

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM

Procedure

6h 35m

1 Wild-type HeLa cells are transiently transfected with pHAGE_TEX264-GFP (RRID:Addgene_201925) or pHAGE_FAM134C-GFP (RRID:Addgene_201927) using Lipofectamine 3000 (Thermo Fisher).

2 After seeding, treat the cells for 06:00:00 with Earle's balanced salt medium starvation medium to induce ER-phagy. 6h

3 After the treatment, collect the cells by trypsinization and wash the cell pellet with PBS once before cells are lysed in lysis buffer.

Lysis buffer:

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM
	NP-40	0.5%

4 Lyse the samples for 00:20:00 On ice before cell lysates are cleared by centrifugation at 20000 x g, 4°C, 00:10:00 . 30m

5 Then, determine the protein concentrations of the cleared protein lysates with the Pierce Detergent Compatible Bradford Assay Kit.

6 Incubate the 6 mg of cell lysate Overnight with 20 µL of GFP-Trap agarose beads.

7 In the morning, wash the samples three times in washing buffer before the beads are resuspended in protein loading dye, supplement with [M] 100 millimolar (mM) DTT, and boil for 00:05:00 at 95 °C . 5m

Washing buffer:

	A	B
	KCl	150 mM
	MgCl2	2.5 mM
	Tris-HCl pH 7.4	20 mM

8 Load the samples on 4-12% SDS-PAGE gels and analyze by western blotting as described above.