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LYSOSOMAL ISOLATION PROTOCOL

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Scott Vermilyea[1]

[1]Univ. of Minnesota, Team Lee

ASAP Collaborative Rese… Team Lee

Jane Balster
ASAP - Team Lee

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

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Abstract

This protocol details the isolation of lysosomes.

Materials

Homogenisation Buffer:

	A	B
	Sucrose	250 mM
	EDTA	2 mM
	Magnesium chloride	1.5 mM
	Potassium Chloride	10 mM
	HEPES	20 mM

TNE lysis buffer:

	A	B
	Tris	50 mM
	NaCl	150 mM
	EDTA, pH 7.4	5 mM
	SDS	1%
	NP-40	0.5%
	DOC	0.5%
	protease/phosphatase inhibitors	

Subcellular Lysosome Isolation Protocol

30m

1 In Vitro Harvest

1.1 Following designated treatment, wash cells three times with DMEM followed by the application of 0.5 mL of homogenization buffer supplemented with proteinase and phosphatase buffer.

Homogenisation Buffer:

	A	B
	Sucrose	250 mM
	EDTA	2 mM
	Magnesium chloride	1.5 mM
	Potassium Chloride	10 mM
	HEPES	20 mM

1.2 Gently detach cells with cell scraper.

1.3 Homogenize using Teflon homogenizer (12 strokes).

1.4 Separate 50 µL of total homogenate (TH) and lyse with *TNE lysis buffer.

TNE lysis buffer:

	A	B
	Tris	50 mM
	NaCl	150 mM
	EDTA, pH 7.4	5 mM
	SDS	1%
	NP-40	0.5%
	DOC	0.5%
	protease/phosphatase inhibitors	

1.5 Centrifuge remaining volume of TH at 1000 x g, 4°C, 00:10:00, collect the supernatant. 10m

1.6 Further centrifuge the supernatant for 20000 x g, 4°C, 00:20:00 to collect the precipitate as crude lysosomal fraction (CLF). Lyse the CLF with *TNE lysis buffer, and prepare lysates for western blot. 20m

TNE lysis buffer:

	A	B
	Tris	50 mM
	NaCl	150 mM
	EDTA, pH 7.4	5 mM
	SDS	1%
	NP-40	0.5%
	DOC	0.5%
	protease/phosphatase inhibitors	