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LYSOSOMAL ISOLATION PROTOCOL

DOI

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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%



A	B
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	