Mar 21, 2025

In Vitro Phosphatase Assay

Nature Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvm9b95l3p/v1

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

DOI: https://dx.doi.org/10.17504/protocols.io.yxmvm9b95l3p/v1

External link: https://doi.org/10.1038/s41556-025-01712-y

Protocol Citation: Elias Adriaenssens 2025. In Vitro Phosphatase Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm9b95l3p/v1

Manuscript citation:
Adriaenssens, E., Schaar, S., Cook, A.S.I. *et al.* Reconstitution of BNIP3/NIX-mitophagy initiation reveals hierarchical flexibility of the autophagy machinery. *Nat Cell Biol* **27**, 1272–1287 (2025). https://doi.org/10.1038/s41556-025-01712-y

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

Created: February 10, 2025

Last Modified: March 21, 2025

Protocol Integer ID: 120012

Keywords: ASAPCRN, vitro phosphatase assay this protocol detail, vitro phosphatase assay, phosphatase assay, assay

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol details the in-vitro phosphatase assay.

Materials

Lysis Buffer:

	A	B
	Tris- HCl, pH 7.4	20 mM
	NaCl	150 mM
	EDTA	1 mM
	EGTA	1 mM
	Triton X-100	1%
	Protease-inhibitor cocktail, but no phosphatase inhibitors	

1x phosphatase buffer:

	A	B
	Tris- HCl, pH 7.4	50 mM
	NaCl	100 mM
	DTT	2 mM
	MnCl2	1 mM

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

Procedure

1h 5m

1 To verify the activity of our recombinantly purified λ phosphatase, collect HeLa cells by trypsinisation and wash the cell pellet with PBS once before cells were lysed in lysis buffer.

Lysis buffer:

	A	B
	Tris- HCl, pH 7.4	20 mM
	NaCl	150 mM
	EDTA	1 mM
	EGTA	1 mM
	Triton X-100	1%
	Protease-inhibitor cocktail, but no phosphatase inhibitors	

2 Lyse the cells for 00:20:00 On ice before cell lysates were cleared by centrifugation at 20000 x g, 4°C, 00:10:00 .

30m

3 Determine the protein concentrations of the cleared protein lysates with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher) and incubate the equal amounts with or without home-made λ phosphatase in 1x phosphatase buffer diluted from a 10x stock.

1x phosphatase buffer:

	A	B
	Tris- HCl, pH 7.4	50 mM
	NaCl	100 mM
	DTT	2 mM
	MnCl2	1 mM

4 For this, protein samples were, where necessary, supplement with lysis buffer to a total volume of 45 µL and supplement with 5 µL of 10x phosphatase buffer.

5 The total amount of protein lysate depends on the abundance of the target protein you want to verify with western blotting. However, for us, this typically ranges between 50 µg - 500 µg in total.

6 Incubate the samples for 00:30:00 at 30 °C before reactions were terminated by the addition of protein loading dye and heat inactivation for 00:05:00 at 95 °C . 35m

7 Analyse the samples by SDS-PAGE and western blotting.

8

Note

Note, some detergents can inhibit the phosphatase reaction, in that case opening the cells with a 26G needle can serve as a substitute in following buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 2 mM DTT).