Sep 23, 2023

In vitro phosphatase assay

DOI
https://dx.doi.org/10.17504/protocols.io.ewov1qyepgr2/v1

Elias Adriaenssens[1]

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

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

DOI: https://dx.doi.org/10.17504/protocols.io.ewov1qyepgr2/v1

Protocol Citation: Elias Adriaenssens 2023. In vitro phosphatase assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1qyepgr2/v1

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

Created: June 27, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 84093

Keywords: In vitro phosphatase assay, ASAPCRN, phosphatase assay, phosphatase, assay this protocol, 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 describes *in vitro* phosphatase assay.

Attachments

PDF

756-1924.pdf
49KB

Materials

Materials

- 6 well plates
- $MnCl_2$

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

Lambda Protein Phosphatase - 100,000 units **New England Biolabs** **Catalog #**P0753L

Lysis buffer

	A	B
	HEPES pH 7.4	50 mM
	NaCl	150 mM
	$MgCl_2$	2.5 mM
	DTT	2 mM
	NP-40	0.50%
	protease inhibitor cocktail	

In vitro phosphatase assay

5m

1 Seed HAP1 wild-type or FIP200 knockout cells in 6 well plates and grow until confluency.

2 Collect cells by trypsinization and pellet by centrifugation at 300 x g, 4°C, 00:05:00 .

5m

3 After a PBS wash to remove the remaining cell medium, resuspend the cell pellets in lysis buffer.

4 Lyse the samples for 00:20:00 On ice . Clear the cell lysates by centrifugation at 20000 x g, 4°C, 00:10:00 .

30m

5 Determine the protein concentrations of the cleared protein lysates with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher).

6 For both samples, wild-type and FIP200 knockout lysates, incubate 100 µg of cell lysate with 5 µL of 10x NEBuffer for Protein MetalloPhosphatases (P0753, New England Biolabs) and 5 µL of [M] 10 millimolar (mM) of $MnCl_2$ to make a total reaction volume of 50 µL .

7 Add 1 µL of Lambda Protein Phosphatase (NEB) to the reaction and incubate the samples at 30 °C for the indicated time.

8 Terminate the phosphatase reactions by the addition of 6x Protein Loading dye and heat inactivation at 95 °C for 00:05:00 .

5m

9 Analyze the samples by western blot analysis as described above.

Protocol references

https://international.neb.com/protocols/2017/12/28/typical-reaction-protocol-for-lambda-protein-phosphatase