In vitro phosphatase assay

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We use this protocol and it's working

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ABSTRACT

This protocol describes *in vitro* phosphatase assay.

ATTACHMENTS

756-1924.pdf

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 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.