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In vitro kinase assay

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We use this protocol and it's working

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Abstract

This protocol describes *in vitro* kinase assay.

Attachments

755-1923.pdf
51KB

Materials

Materials

- Recombinant proteins TBK1, ULK1 complex, and NAP1
- $MgCl_2$
- ATP
- dH_2O
- Nitrocellulose membranes (RPN132D, GE Healthcare)
- Mini Trans-Blot Cell (Bio-Rad).
- SDS-PAGE gels (NP0321BOX, NP0322BOX, or NP0323BOX, Thermo Fisher)
- PageRuler Prestained protein marker (Thermo Fisher)

Kinase buffer

	A	B
	Tris-HCl pH 7.4	20 mM
	NaCl	150 mM
	DTT	1 mM

Fixation solution

	A
	40% ethanol
	10% acetic acid
	50% dH_2O

In vitro kinase assay

25m

1 Mix recombinant proteins TBK1 or ULK1-complex (composed of ULK1, FIP200, ATG13, and ATG101) and NAP1 in kinase buffer.

2 Use the kinases at [M] 50 nanomolar (nM) and mix with [M] 250 nanomolar (nM) NAP1.

3 Start the kinase reactions by the adding 2x ATP/$MgCl_2$ kinase buffer to a final concentration of [M] 10 millimolar (mM) $MgCl_2$ and [M] 100 millimolar (mM) ATP.

4 Prepare protein mixtures as master mixes and divide over the number of time points.

5 To control for potential protein instability, induce the latest time point first and then go gradually to the shortest time point.

6 In this way, keep all protein mixtures at Room temperature for the same time, and terminate the reactions together.

7 Achieve the termination of reactions by the addition of 6x Protein Loading dye and heat inactivation at 95 °C for 00:05:00 .

5m

8 Separate the samples on 4-12% SDS-PAGE gels (NP0321BOX, NP0322BOX, or NP0323BOX, Thermo Fisher) with PageRuler Prestained protein marker (Thermo Fisher).

9 After the run, either stain the SDS-PAGE gel with Coomassie or transfer to nitrocellulose membranes for western blot analysis.

10 In the case of Coomassie staining, incubate the gel for 00:10:00 in Coomassie solution, fix for 00:10:00 with fixation solution, and then destain it Overnight in dH_2O.

30m

11 Cut the band corresponding to NAP1 from the gel with a fresh scalpel and submit for mass spectrometry analysis.

12 In the case of western blotting, transfer the proteins onto nitrocellulose membranes (RPN132D, GE Healthcare) for 01:00:00 at 4 °C using the Mini Trans-Blot Cell (Bio-Rad). 1h

13 Process the membranes further for western blot analysis, as described in the western blot protocol.