

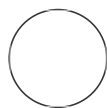


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## In vitro kinase assay

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### ABSTRACT

This protocol describes *in vitro* kinase assay.

### ATTACHMENTS

[755-1923.pdf](#)

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

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**Keywords:** in vitro kinase assay

MATERIALS

**Materials**

- Recombinant proteins TBK1, ULK1 complex, and NAP1
- MgCl<sub>2</sub>
- ATP
- dH<sub>2</sub>O
- 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 <sub>2</sub> O

*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<sub>2</sub> kinase buffer to a final concentration of

[M] 10 millimolar (mM)  $\text{MgCl}_2$  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  $\text{dH}_2\text{O}$ .

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.