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

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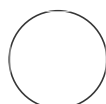
## In vitro kinase activity

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### ABSTRACT

This protocol details methods for the *in vitro* kinase activity testing of purified LRRK2.

### ATTACHMENTS

[iuuabvk9p.docx](#)

### MATERIALS

-  SuperSep Phos-tag Gel Fujifilm Wako Pure Chemical Catalog #195-1799
- Anti-LRRK2 (phospho T1357)
-  Anti-LRRK2 (phospho T1357) antibody Abcam Catalog #ab270606

### Solutions to prepare:











#### 10x Kinase buffer:

A	B
Tris-HCl (pH 7.5)	200 mM
MgCl <sub>2</sub>	75 mM
EGTA	1 mM

**Keywords:** Kinase activity,  
In vitro, LRRK2

## In vitro kinase activity

2h 10m

-  Set up the reaction mixtures with 1x kinase buffer (diluted from 10x kinase buffer),  
 200 nanomolar (nM) LRRK2 or  8  $\mu\text{g}$  Rab8 protein, for the reaction group add  
 1 millimolar (mM) ATP, and for the control add H<sub>2</sub>O instead, the total volume we used is  
 40  $\mu\text{L}$  .
-  Incubate samples for  02:00:00 at  30 °C . 2h
- Quench reactions through addition of SDS sample loading buffer and heat at  95 °C for 10m  
 00:10:00 .
- Resolve samples by SDS/PAGE or Phos-tag SDS/PAGE.
- Detect proteins by coomassie blue staining or western blot using antibodies of Rab8 and LRRK2 phospho-specific (pT1357), respectively.