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In vitro kinase activity

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Attachments



[iuuabvk9p.docx](#)

17KB

Materials

- SuperSep Phos-tag Gel **FUJIFILM Wako Pure Chemical Corporation 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 ₂	75 mM
	EGTA	1 mM



In vitro kinase activity

2h 10m

- 1 Set up the reaction mixtures with 1x kinase buffer (diluted from 10x kinase buffer),
[M] 200 nanomolar (nM) LRRK2 or  8 μ g Rab8 protein, for the reaction group add
[M] 1 millimolar (mM) ATP, and for the control add H₂O instead, the total volume we
used is  40 μ L . 
- 2 Incubate samples for  02:00:00 at  30 °C . 
- 3 Quench reactions through addition of SDS sample loading buffer and heat at  95 °C
for  00:10:00 . 
- 4 Resolve samples by SDS/PAGE or Phos-tag SDS/PAGE.
- 5 Detect proteins by coomassie blue staining or western blot using antibodies of Rab8 and LRRK2 phospho-specific (pT1357), respectively.