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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
	MgCl2	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 µg 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 µL .

2 Incubate samples for 02:00:00 at 30 °C . 2h

3 Quench reactions through addition of SDS sample loading buffer and heat at 95 °C for 00:10:00 . 10m

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.