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In vitro GTPase activity

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We use this protocol and it's working

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Abstract

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

Attachments

DOCX

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16KB

Materials

- EnzChek™ Phosphate Assay Kit **Thermo Fisher Catalog #**E6646
- 96-well Clear Flat Bottom Polystyrene TC-treated Microplate Individually Wrapped with Low Evaporat **Corning Catalog #**3595
- Microplate reader (Synergy H1; BioTek)

In vitro GTPase activity

1h 16m

1 Set up the reaction mixtures in a 96-well plate with 5 µL 20× reaction buffer ([M] 1 Mass Percent Tris-HCl, [M] 20 millimolar (mM) MgCl2, pH 7.5 and [M] 2 millimolar (mM) sodium azide), [M] 200 micromolar (µM) 2-amino-6-mercapto-7-methylpurine riboside, 0.1 U purine nucleoside phosphorylase, and [M] 9 micromolar (µM) LRRK2 protein or [M] 0.8 micromolar (µM) Dynamin1 or buffers for the control in separate wells.

Note

Note: For best measurement results, we usually use a total volume of 80 µL - 100 µL .

2 Preincubate samples in the microplate reader for 00:30:00 at 37 °C . 30m

3 Add [M] 0.5 millimolar (mM) GTP (final concentration) to initiate the reaction.

4 Immediately begin reading absorbance at 360 nm , every 00:01:00 over 00:45:00 at 37 °C . 46m

5 For data analysis, subtract the last values determined before GTP was added from the corresponding values for the experimental reaction.