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

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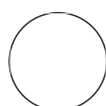
## In vitro GTPase activity

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### ABSTRACT

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

### ATTACHMENTS









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### 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  $\mu\text{L}$  20 $\times$  reaction buffer ( 1 Molarity (M) Tris-HCl,  20 millimolar (mM)  $\text{MgCl}_2$ ,  7.5 and  2 millimolar (mM) sodium azide),  200 micromolar ( $\mu\text{M}$ ) 2-amino-6-mercapto-7-methylpurine riboside, 0.1 U purine nucleoside phosphorylase, and  9 micromolar ( $\mu\text{M}$ ) LRRK2 protein or  0.8 micromolar ( $\mu\text{M}$ ) Dynamin1 or buffers for the control in separate wells.





### Note

**Note:** For best measurement results, we usually use a total volume of  80  $\mu\text{L}$  -  100  $\mu\text{L}$ .

- 2 Preincubate samples in the microplate reader for  00:30:00 at  37  $^{\circ}\text{C}$ .

30m

- 3 Add  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  $^{\circ}\text{C}$ .

46m

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