***In vitro* GTPase activity**

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

Materials and Equipment:

EnzChek Phosphate Assay Kit (Invitrogen, E6646), 96-well plate (Corning, 3595), Microplate reader (Synergy H1; BioTek).

**Protocol:**

1, Set up the reaction mixtures in a 96-well plate with 5μl 20× reaction buffer (1 M Tris-HCl, 20 mM MgCl2, pH7.5 and 2 mM sodium azide), 200 μM 2-amino-6-mercapto-7-methylpurine riboside, 0.1 U purine nucleoside phosphorylase, and 9 μM LRRK2 protein or 0.8 μM Dynamin1 or buffers for the control in separate wells.

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

2, Preincubate samples in the Microplate reader for 30 min at 37°C.

3, Add 0.5 mM GTP (final concentration) to initiate the reaction.

4, Immediately begin reading absorbance at 360 nm, every 1 min over 45 min at 37°C.

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