***In vitro* GTPase activity**

**Authors:** Xinbo Wang1,2 and Pietro De Camilli1,2

1Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
2Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

**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.