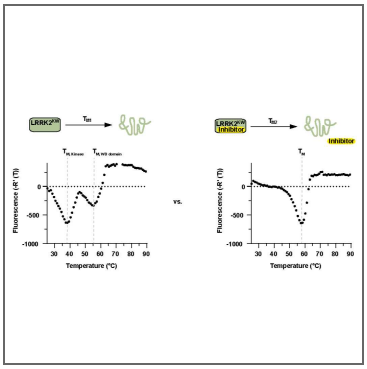

VERSION 1

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

LRRK2 thermal shift assay V.1

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ABSTRACT

Thermal shift assay or differential scanning fluorimetry analyzes the effect of small molecules on the thermostability of a protein by gradual heat denaturation and monitoring absorption of the fluorescent dye SYPR Orange at 488 nm.

MATERIALS

Thermal shift buffer

20 mM Hepes pH 7.4
150 mM NaCl
5% glycerol

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PROTOCOL integer ID:
89851

Fluorescent-based thermal shift assay

1 Prepare 4 µM master mix of protein in buffer (20 mM Hepes pH 7.4, 150 mM NaCl, 5% glycerol) and add 1:1000 dilution of SYPR Orange.

2 Aliquot 20 µL of the master mix into a white 96 well plate.

3 Add DMSO or small molecule binder with a final concentration of 10 µM.

4 Seal plate, mix well and centrifuge 30 sec at 500xg.

5 Place plate into MX3005P real-time PCR instrument.

6 Measure fluorescence with excitation and emission filters set to 465 and 590 nm while gradually increase temperature 3K/min during 71 cycles from 25 to 95°C.