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LRRK2 thermal shift assay V.1

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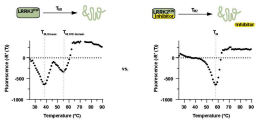

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Protocol status: Working
We use this protocol and it's working

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Keywords: thermal shift assay, lrrk2 thermal shift, absorption of the fluorescent dye sypr orange, thermal shift, fluorescent dye sypr orange, protein, thermostability, scanning fluorimetry, gradual heat denaturation, lrrk2, small molecule, effect of small molecule, molecule, monitoring absorption

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

Troubleshooting

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.