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Version 2

LRRK2 thermal shift assay V.2

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We use this protocol and it's working

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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.



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.