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LRRK2 PhosphoSens Assay V.2

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We use this protocol and it's working

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Abstract

With this enzymatic activity assay we were able to determine IC₅₀ values of published inhibitors as well as newly synthesized compounds that inhibit LRRK2. The assay is based on the in vitro phosphorylation reaction between the enzymatic active LRRK2(RCKW)-construct and the SOX-based substrate peptide (AQT0615 from AssayQuant Technologies).



- 1 Make 10mM, 100 μ M and 1.6 μ M stock solutions of the compounds in DMSO. 1h
- 2 Pipett a dilution series of eleven concentrations between 15 μ M and 0.4nM (calculated with an assay volume of 10 μ L) of the compounds into white 384-well plates (Greiner 781207) as duplicates with an ECHO acoustic dispenser (Labcyte). Pipett a equivalent of DMSO in two wells per compound as 0% (without protein and compound) and 100%(without compound) control. 20m
- 3 Dilute the purified LRRK2^{RCKW} to 22nM with the reaction buffer (50mM HEPES buffer (pH7.5), 10mM MgCl₂, 1% glycerol, 1mM DTT, 0.2mg/mL BSA, 0.01% Tween20, 5 μ M AQT0615) and add 10 μ L to each well, except the 0% control wells, with a multichannel pipette (Eppendorf). Add 10 μ L of pure reaction buffer to the 0% control wells. 20m
- 4 Pipett 5nL 100mM ATP stock into each well with the ECHO acoustic dispenser (Labcyte). 10m
- 5 Centrifuge the plates at 1500g_n for 2min, before incubating the reaction at room temperature for 4h. 4h
- 6 Measure the fluorescence after 360nm excitation at 487nm emission with a PHERAstar plate reader (BMG Labtech). 10m
- 7 Normalize the response to the average 0% and 100% controls. Calculate the IC₅₀ values via non-linear regression of the log[inhibitor] vs. the normalized response with GraphPad Prism 8. 30m