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Kinase activity assays Src and CK2

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We use this protocol and it's working

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Abstract

This protocol details about the Kinase activity assays of Src and CK2.

1 To verify the activity of kinases SRC and CK2, add 45 µL of mixes containing either only kinase assay buffer ([M] 25 millimolar (mM) Tris-HCl pH 7.4 , [M] 150 millimolar (mM) NaCl, [M] 1 millimolar (mM) DTT, and [M] 2 millimolar (mM) $MgCl_2$), kinase buffer and substrate ([M] 0.5 mg/mL) or kinase buffer, substrate ([M] 0.5 mg/mL) and kinase ([M] 100 nanomolar (nM)) to individual wells of a Pierce white opaque 96-well plate (Thermo Scientific).

2 Substrate peptides used were RRRDDDSDDD 10-mer (PEP-CK2I-025, Biaffin) and Poly-(Glu,Tyr 4:1) (40217, BPS) for CK2 and Src kinases, respectively. For CK2, add a specific inhibitor Silmitasertib CX-4945 (S2248, Selleck- chem), where indicated, at a concentration of [M] 1 micromolar (µM) .

3 Start the reactions by the addition of 5 µL ATP in kinase assay buffer, resulting in a final concentration of [M] 100 micromolar (µM) ATP in each of the 50 µL reactions.

4 After 01:00:00 at Room temperature (RT) in darkness, add 50 µL of Kinase-Glo Max reagent (Promega) to each well, to reach a total volume of 100 µL . 1h

5 Allow the luciferase reactions to stabilize for 00:15:00 before measuring luciferase activity at a Spark Multi-Mode Microplate Reader (TECAN). The luciferase activity correlates with ATP quantity, and thus, an inverse relationship between measured luminescence and kinase activity exists. 15m