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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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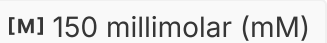
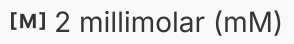
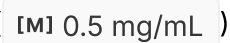
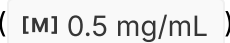
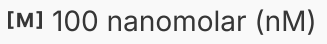
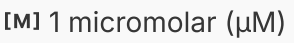
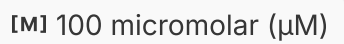
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Abstract

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 ( 25 millimolar (mM) Tris-HCl  7.4 ,  150 millimolar (mM) NaCl,  1 millimolar (mM) DTT, and  2 millimolar (mM) MgCl_2), kinase buffer and substrate ( 0.5 mg/mL) or kinase buffer, substrate ( 0.5 mg/mL) and kinase ( 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  1 micromolar (μM) . 
- 3 Start the reactions by the addition of  5 μL ATP in kinase assay buffer, resulting in a final concentration of  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