ab283366 – CSK Inhibitor Screening Kit (Fluorometric)

For the screening of potential CSK inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283366

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Src Assay Buffer	25 mL	-80°C
Fluorogenic Probe	200 µl	-80°C
ADP Detection Mix	1 vial	-80°C
Developer Enzyme Mix	1 vial	-80°C
Src Peptide Substrate	1 vial	-80°C
Ultra-Pure ATP	1 vial	-80°C
Recombinant Human Src	100 µl	-80°C
Dasatinib (1mM)	50 µl	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well fluorescence microplate reader
- Black 96-well plate with flat bottom

Reagent Preparation

- Prior to use, store kit at -80°C and protect from light. Briefly centrifuge all small vials prior to opening.

Src Assay Buffer: Warm to room temperature (RT) before use. Store protected from light.

Fluorogenic Probe (in DMSO): Aliquot and store in the dark. Thaw at room temperature before use. After use, promptly retighten cap to minimize adsorption of airborne moisture.

ADP Detection Mix and Developer Enzyme Mix: Reconstitute each vial with 220 µl Src Assay Buffer. Aliquot as desired and keep protected from light. Avoid repeated freeze/thaw cycles.

Src Peptide Substrate: Reconstitute with 220 µl ddH_2O to obtain a 50X stock solution. Aliquot as desired and keep protected from light. Stable for 3 freeze/thaw cycles.

Ultra-Pure ATP: Reconstitute with 220 µl $ddH_2O$to obtain a 12.5 mM stock solution. Aliquot as desired. Avoid repeated freeze/thaw cycles.

Recombinant Human Src: Aliquot as desired. Avoid repeated freeze/thaw cycles.

Dasatinib (1 mM): Provided as a solution in DMSO. Allow to thaw to RT before use. Stable for 3 freeze/thaw cycles.

Δ Note: *Keep* Recombinant Human c-Src and Ultra-Pure ATP *on ice while performing the assay.*

Assay Protocol

Test Compound preparation:

1. Dissolve each test compound (TC) in appropriate solvent to produce stock solution.
2. Prepare 10X working solution by diluting stock solution in Src Assay Buffer.
3. To determine IC_{50} values for TC's, 10X solutions should be prepared in a range of concentrations in order to generate a multi-point dose-response curve (the concentration of organic solvent should be the same for all test concentrations).
4. Organic solvent concentration should be minimized to avoid impacting enzyme activity (DMSO has little effect on c-Src kinase activity at a final concentration of ≤1%).
5. For higher concentrations or solvents other than DMSO, recommended to prepare a Vehicle Control (VC) well with the same final concentration of the solvent used to solubilize TCs. Use the VC well to define 100% activity if it is different from the "No Inhibitor Control well(s)".

Reaction Mix:

1. Prepare a diluted c-Src enzyme solution by diluting Recombinant Human Src stock in Src Assay Buffer at a 1:10 ratio (for example, for 10 reactions, mix 10 µl of Recombinant Human Src with 90 µl Src Assay Buffer). Make enough diluted Src enzyme to add 10 µl to each reaction well.

Δ Note: *Remember to account for No Inhibitor Control/Vehicle Control and Reference Inhibitor Control wells when calculating the amount of diluted enzyme to prepare.*

2. Set up the Assay Reactions according to the table below. Prepare reaction wells containing Test Compound(s), as well as corresponding No Inhibitor Control (which may also serve as the Vehicle Control (VC), if desired) and Background Control (containing no kinase). A Reference Inhibitor Control well may also be prepared using the included inhibitor Dasatinib. Dilute the 1 mM Dasatinib stock solution at a 1:100 ratio (add 10 µl of the 1 mM solution to 990 µl Src Assay Buffer), yielding a 10 µM Dasatinib working solution and add 10 µl of the working solution to each Reference Inhibitor Control well. Adjust the volume of all wells to 60 µl with Src Assay Buffer.

	No Inhibitor/VC	+Test Compound	Reference Inhibitor	Background
Diluted Src Enzyme	10 µl	10 µl	10 µl	-
Test Compound (10X)	-	10 µl	-	-
Dasatinib (10 µM)	-	-	10 µl	-
Src Assay Buffer	50 µl	40 µl	40 µl	60 µl

Δ Note: *For Vehicle Control (VC) well, use 50 µl Src Assay Buffer containing the test compound solvent at 2X final well concentration.*

3. Pre-incubate the plate for 15 minutes at 37°C to allow Test Compound(s) to interact with Src. During the pre-incubation, prepare Reaction Mix according to the table below. Make a sufficient amount of Reaction Mix to add 40 µl to each reaction well.

	Reaction Mix
ADP Detection Mix	2 µl
Developer Enzyme Mix	2 µl
Ultra-Pure ATP	2 µl
Src Peptide Substrate	2 µl
Fluorogenic Probe	1 µl
Src Assay Buffer	31 µl

Start the reaction by adding 40 µl of Reaction Mix to all reaction wells, yielding a final reaction volume of 100 µl/well.

Measurement

Measure the fluorescence at Ex/Em = 535/587 nm in kinetic mode at 37°C for 30-45 minutes. While the assay can be performed in either endpoint or kinetic mode, we strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction.

Calculation:

1. For each reaction well (including Background and No Inhibitor/Vehicle Control wells), choose any two time points (T1 and T2) in the linear phase of the reaction progress curve. Obtain the corresponding fluorescence values at those points (RFU1 and RFU2) and determine:

   $$\Delta F = (RFU2 - RFU1) \text{ and } \Delta T = (T2 - T1)$$

2. Calculate the Background-corrected reaction rate (denoted by R) for each well by subtracting the rate of the Background Control (ΔFBC) reaction from each:

   $$R = (\Delta F - \Delta FBC) / \Delta T$$

3. Calculate the percent inhibition due to the Test Compound or Reference Inhibitor Control using the following equation (where RVC represents the Background-corrected rate of the No Inhibitor/Vehicle Control condition):

   $$\text{\% Relative inhibition} = \frac{Rvc - RTc}{Rvc} \times 100\%$$

Technical Support

For all technical or commercial enquiries please go to:

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