QS S Assist STK_FP Kit

Description

STK FP kit is designed for use in pharmacological assays for STK based on fluorescence polarization. The kit includes assay buffer, human protein kinase, ATP/fluorescence- labeled substrate peptide and a protocol to perform 384 well plate assays.

Components (800 dp)

Materials	Volume	Storage
10 x Assay Buffer	5 mL	-80°C
600 x STK	30 µL	-80°C
5 x ATP/FITC-labeled substrate peptide	1 mL	-80°C (light shielding)

Please avoid repeated freeze-thaw cycles.

Reagent Preparation (per 400 dp)

Bring all reagents (except kinases) to room temperature before use.

Materials provided

Assay Buffer

Thaw Assay Buffer (10 x) and dilute 2 mL of Assay Buffer (10 x) with 18 mL of distilled water. The Assay Buffer is kept at room temperature before use. Please do not carry over this buffer on the next day, because the buffer component DTT is unstable.

ATP/Substrate Solution

Thaw ATP/FITC-labeled substrate peptide (5 x) and 5-fold dilute it with Assay Buffer. This ATP/ FITC-labeled substrate peptide reagent includes an appropriate concentration of $MgCl_2$ or $MnCl_2$. ATP/Substrate Solution is kept at room temperature with light shielding until use.

Enzyme Solution

Thaw STK (600 x) and 600-fold dilute it with Assay Buffer. Please keep the enzyme solution on ice until use.

Materials required

Compound Solution

Prepare 100-times higher concentration of compound solution with DMSO. Dilute each compound solution 25 times with Assay Buffer. For the vehicle, prepare 4% DMSO-Assay Buffer solution.

Detection Mixture

Please prepare IMAP™ Screening Express Kit (Progressive Binding System), R8127, Molecular Devices Corporation.

For one plate (384 wells) determination, 5-fold dilute Binding Buffer A (R7282) and Binding Buffer B (R7283) with distilled water, and mix Binding Buffer A: Binding Buffer B = 100:0. Add 75 µL of IMAP™ Binding Reagent to the 29.925 mL of Binding Buffer (400-fold dilution). Allow the Detection Mixture place at room temperature until use.

Example of Reagent Preparation

Reagent	Preparation
Assay Buffer	10 x Assay Buffer, 2 mL + distilled water 18 mL
Kinase	600 x STK, 10 µL + Assay Buffer, 5990 µL
ATP/Substrate	5 x ATP/Substrate, 0.5 mL + Assay Buffer, 2.0 mL
Detection Mixture	IMAP™ Binding Reagent, 75 µL + Binding Buffer, 29.925 mL

Example of Reaction

Sample	Compound solution (µL)	Vehicle (µL)	ATP/Substrate (µL)	Enzyme (µL)	Assay Buffer (µL)
A	—	5	5	—	10
B	—	5	5	10	—
C	5	—	5	10	—

Calculation of inhibition by compound (%)

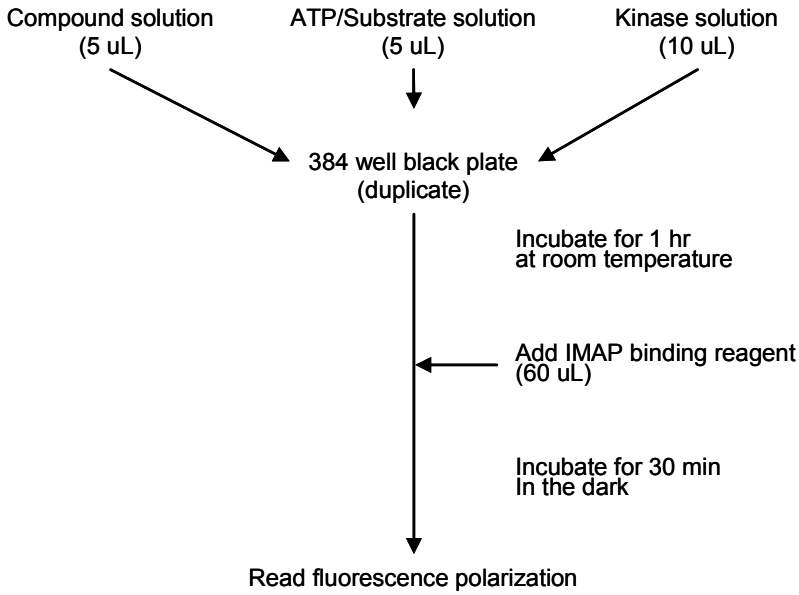
Inhibition (%)＝(1－(C－A)/(B－A))x100

Final Concentrations of Components in Reaction Mixture

20 mM HEPES (pH7.4), 0.01% Tween20, 2 mM DTT

100 nM substrate, 25 µM ATP, 5 mM Mg

Illustration of Assay Procedures:



ASSAY PROCEDURE:

All procedures are performed at room temperature.

1. Add 5 μL of vehicle (4% DMSO) to wells of "A" and "B" and compound solution to well of "C".
2. Add 5 μL of ATP/Substrate solution to each well.
3. Add 10 μL of Assay Buffer to well of "A" and enzyme solution to well of "B" and "C" to start kinase reaction. Cover the plate and incubate for one hour.
4. Add 60 μL of IMAP™ Detection Mixture to stop kinase reaction. Incubate for 30 minutes at room temperature.
5. Measure fluorescence polarization (mP values) with a plate reader (excitation 485 nm, emission 530 nm).
6. Calculate of inhibition percentage of compound as follows; Inhibition (%)＝(1－(C－A)/(B－A)) x 100

The settings for the instrument (Analyst AD, Molecular Devices Corporation)

Parameter	**Setting**
Detection mode	Fluorescence Polarization
Lamp	Continuous
Plate format	Corning 384 Square Opaque PS
Switch Polarization	By Well
G Factor	0.96755
Z Height	Middle of well (= 5.775 mm, Corning, 3710)
Raw data units	Counts/sec
Excitation	Fluorescein 485 nm
Emission	Fluorescein 530 nm
Readings per well	1
Integration time	100,000 us
Attenuator mode	Out

Dynamic polarizer	Emission
Static polarizer	S
Shaking Time	0 sec, Medium
Plate setting time	25 ms
PMT Setup	SmartRead, Sensitivity 2
Delay before first read	0 sec
Delay between reads	0 sec
Number of reads	1

Assay result example

The inhibitory effect of Staurosporine on STK evaluated with STK FP kit is shown below.