QS S Assist KINASE_TR-FRET Kit

Description

KINASE TR-FRET kit is designed for use in pharmacological assays for KINASE based on Time-Resolved Fluorescence Resonance Energy Transfer. The kit includes assay buffer, human protein kinase, ATP/Biotinylated substrate peptide, and a protocol to perform 384 well plate assays.

Components (400 dp x 2)

Materials	Volume	Storage
10 x Assay Buffer	10 mL	-80°C
30000 x KINASE	60 µL	-80°C
5 x ATP/Biotinylated substrate Peptide	2 mL	-80°C

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 take 2 mL. Dilute with 18 mL of distilled water. Adjusted Assay Buffer is able to keep 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/Biotinylated substrate peptide (5 x) and 5-fold dilute it with Assay Buffer. This ATP/Biotinylated 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

Thaw KINASE (30000 x) and 30000-fold dilute it with Assay Buffer. Please keep the enzyme on ice before use.

Materials required

Compound Solution

Prepare a hundred times concentrated compound stock solution with DMSO. Dilute the solution 25 times with the Assay Buffer. For the vehicle control, prepare 4% DMSO-Assay Buffer solution.

Detection Mixture

Prepare Detection Mixture containing appropriate concentrations of EDTA, Eu-labeled Antibody (LANCE Eu-W1024 anti-phosphotyrosine (PT66), AD0069, PerkinElmer), and Streptavidine-Allophycocyanin (SureLight™ Allophycocyanin-streptavidin (APC-SA), CR130-100) in Detection Buffer (15 mM Tris-HCl (pH7.5), 0.01% Tween20). Detection Mixture is able to keep room temperature before use with light shielding.
Minimal 24 mL of Detection Mixture is needed for 400 dp (60 µL/well).

The final concentrations of EDTA, Eu-labeled Antibody, and SA-APC in the detection mixture is 20 mM, 0.53 nM, and 33.3 nM, respectively in the protocol.

Summary of Reagent Preparation

Reagent	Preparation
Assay Buffer	Assay Buffer (10 x), 2 mL + distilled water, 18 mL
Kinase	KINASE (30000 x), 5 µL + Assay Buffer, 145 µL to make KINASE (1000 x) KINASE (1000 x) , 5 µL + Assay Buffer, 4.995 mL
ATP/Substrate	ATP/Substrate (5 x), 0.5 mL + Assay Buffer, 2.0 mL

Example of Reaction Mixture

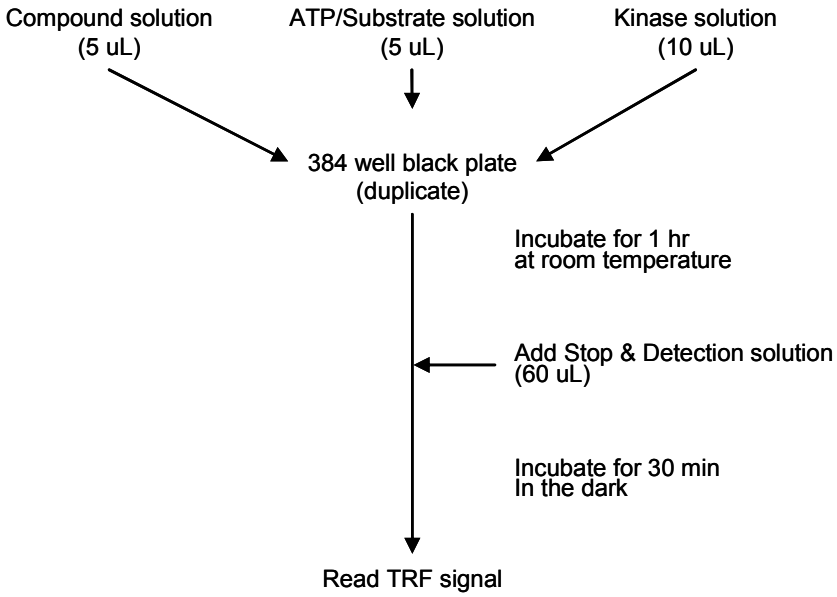
Sample	Compound solution (µL)	Vehicle (µL)	ATP/Substrate (µL)	Enzyme (µL)	Assay Buffer (µL)
A	–	5	5	–	10
B	–	5	5	10	–
C	5	–	5	10	–

Calculation of inhibition by compound (%)
Inhibition (%)=(1−(C−A)/(B−A)) x 100

Final Concentrations of Components in Reaction Mixture

15 mM Tris-HCl (pH7.5), 0.01% Tween20, 2 mM DTT
250 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 Compound Solution, 5 µL of ATP/Substrate solution to the assay plate, and then add 10 µL of enzyme solution to start kinase reaction. Incubate for 1 hour.

2. Add 60 µL of Detection Mixture. Incubate for 30 min at room temperature.

3. Measure TR-FRET signal with a plate reader (excitation 360 nm, emission 665 nm).

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

Parameter	Setting
Detection mode	TR-FRET[Eu-APC]
Optics	Top
Plate format	Corning 384 Square Opaque PS
Z Height	5.77 mm
Raw data units	Counts
Excitation	Europium 360 nm
Emission	APC/Cy5 665 nm
Flashes per well	100
Integration time	500 µs
Interval between Flashes	1 x 10 ms
Delay after flash	60 µs
Attenuator mode	Out

Shaking Time	0 sec, Medium
Plate setting time	25 ms
PMT Setup	Digital, Sensitivity 2
Delay before first read	0 sec
Delay between reads	0 sec
Number of reads	1
Flash lamp voltage	1000 Volts

We recommend you to measure Europium signal (Emission; 620 nm) to confirm assay accuracy.

Assay result example

The inhibitory effect of Staurosporine on KINASE evaluated with KINASE TR-FRET kit is shown below.