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 (TR-FRET). The kit includes assay buffer, human protein kinase, ATP/Biotinylated substrate peptide/Metal and a protocol to perform 384 well plate assay.

Components (800 dpt x 1 set)

Materials	Volume	Storage
10 x Assay Buffer	5 mL x 1	-80°C
KINASE *	60 µL x 1	-80°C
5 x ATP/Substrate/Metal	1 mL x 1	-80°C

* Concentration of the kinase; 200 µg/mL

Please avoid repeated freeze-thaw cycles.

Reagent Preparation (per 400 dpt)

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

Materials provided

Assay Buffer

Thaw 10 x Assay Buffer 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/Metal Solution

Thaw 5 x ATP/Substrate/Metal component and dilute 0.45 mL into 1.8 mL of Assay Buffer (total volume: 2.25 mL). Bring the solution to room temperature until use.

Enzyme Solution

Thaw KINASE and dilute it appropriate-fold with Assay Buffer. Please keep it on ice before use.

Materials required

Compound Solution

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

Detection Mixture

Prepare the Detection Mixture containing appropriate concentrations of Tris-HCl (pH7.5), Tween20, EDTA, Eu-labeled Antibody, and Streptavidin-Allophycocyanin in distilled water. The Detection Mixture is able to keep room temperature before use with light shielding.
Minimal 24 mL of the Detection Mixture is needed for 400 dpt (60 µL/well).

The final concentrations of Tris-HCl (pH7.5), Tween20, EDTA, Eu-labeled Antibody, and Streptavidin-Allophycocyanin in the detection mixture is 15 mM, 0.01 %, 20 mM, 0.53 nM, and 33.3 nM (as measured by Streptavidin concentration), respectively in the protocol.

Example of Reaction Mixture

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

Where A equals negative control, B equals positive control and C equals test sample.
Calculate the percent inhibition of compound as follows;
Inhibition (%)＝(1－(C－A)/(B－A)) x 100

Final Concentrations of Components in Reaction Mixture

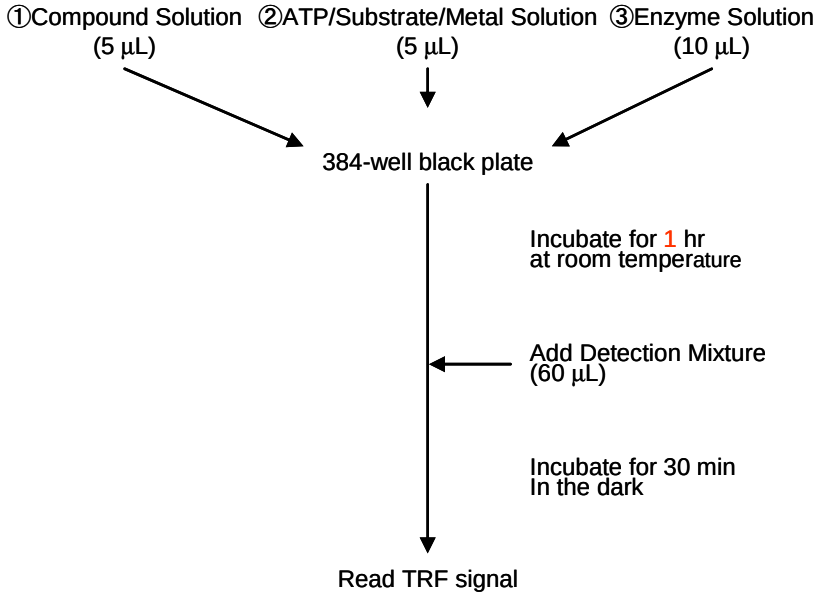
15 mM Tris-HCl (pH7.5), 0.01 % Tween 20, 2 mM DTT
250 nM Biotinylated substrate peptide, 5 µM ATP, 5 mM Metal

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 wells of , "C" of a 384-well assay plate.
2. Add 5 µL of ATP/Substrate/Metal Solution to each well.
3. Add 10 µL of Assay Buffer to wells of "A" and Enzyme Solution to wells of "B" and "C" to start kinase reaction. Cover the plate and incubate for **1** hour at room temperature.
4. Add 60 µL of Detection Mixture to each well. Incubate for 30 minutes at room temperature with light shielding.
5. Measure the TR-FRET signal with a plate reader (excitation 360 nm, emission 665 nm).

Illustration of Assay Procedures:



The settings for the instrument (2104EnVision D, PerkinElmer)

Parameter	**Setting**
Light source	Laser
Top mirror	LANCE/DELFIA Bias
Excitation filter	TRF 320nm
Emission filter	APC 665nm
Emission filter 2	Europium 615nm
Measurement height	7 mm
Cycle	16600
Delay	50 µs
Number of flashes	40
Window time	400 µs

Assay result example

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