QS S Assist KINASE_MSA Kit

Description

KINASE MSA Kit is designed for use in pharmacological assays for KINASE based on Off-chip mobility shift assay. This kit includes assay buffer concentrate (10 x), termination buffer concentrate (4 x), human protein kinase, ATP/substrate/metal concentrate (5 x) and a protocol to perform assay.

Components

Materials	Volume	Storage
10 x Assay Buffer	10 mL	-80°C
4 x Termination Buffer	15 mL	-80°C
KINASE *	25 µL	-80°C
5 x ATP/substrate/metal	1.0 mL	-80°C (light shielding)

* Concentration of the kinase; 200 µg/mL

Materials provided

Assay Buffer Concentrate

Thaw 10 x Assay Buffer and dilute 5 mL into 45 mL of distilled water (total volume: 50 mL). Hold diluted assay buffer at room temperature, discarded unused portion at end of day due to instability of DTT in this component.

Termination Buffer Concentrate

Thaw 4 x Termination Buffer and dilute 5 mL into 15 mL of distilled water (total volume: 20 mL). Bring termination buffer to room temperature (25±2℃) prior to use.

ATP/substrate/metal Solution Concentrate

Thaw 5 x ATP/substrate/metal solution and dilute 0.9 mL into 3.6 mL of distilled water (total volume: 4.5 mL). Bring the solution to room temperature and shield from light until use.

Enzyme Solution

Thaw KINASE (200 µg/mL) and make aliquots, if the kinase is not used at once. Dilute it appropriate-fold with Assay Buffer (please refer to recommended dilution on page 2). Keep the enzyme solution on ice until use.

Avoid further repeated freeze-thaw cycles for aliquots.

Materials required

Sample Preparation

Prepare 100 x concentrate of compound solution in DMSO. Dilute each compound solution 25 x into assay buffer (creates 4% concentrate of sample). Assay buffer containing 4% DMSO (no sample) is used for vehicle control.

Separation Buffer (Purchase Coating-3 Reagent from Caliper LS, cat. no. 760050)
100 mM HEPES pH7.5, 10 mM MgCl2, 0.01% Triton X-100, 10 mM EDTA-2Na, 1% DMSO, 0.1% Coating-3 Reagent.

Example of Reaction

Sample	Compound solution (μL)	Vehicle (μL)	ATP/Substrate (μL)	Enzyme (μL)	Assay Buffer (μL)
Negative Control	—	5	5	—	10
Positive Control	—	5	5	10	—
Test	5	—	5	10	—

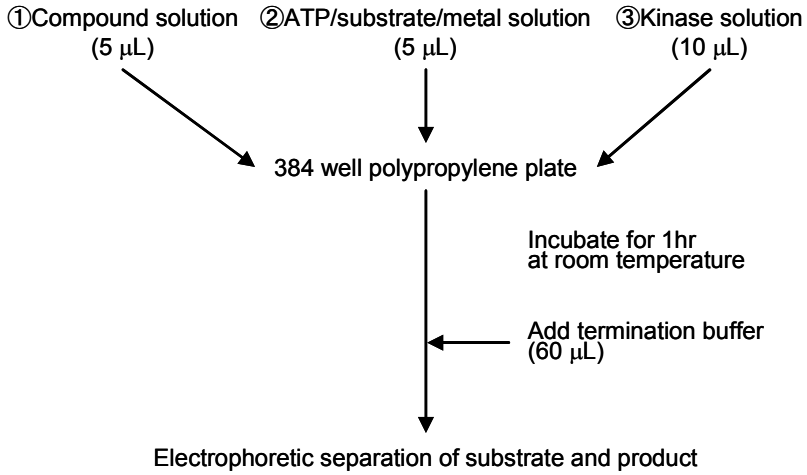
Calculation of inhibition by compound (%)
Inhibition (%)＝(1－(Test－Negative Control)/(Positive Control－Negative Control))x100

Final Concentrations of ATP/substrate/metal in reaction mixture at 20 μL/well:
50 μM ATP, 1 μM substrate, and 5 mM $MgCl_2$

Recommended dilution of KINASE with Assay Buffer
1000-fold (final 2000-fold dilution of the kinase in the reaction mixture (20 μL/well))
(P/(P+S) = 0.3-0.5 under the recommended condition)

Reaction time and temperature
1 hour at room temperature (25±2℃)

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",
   where A equals negative control, B equals positive control and C equals test sample.
2. Add 5 μL of ATP/substrate/metal 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 1 hour.
4. Add 60 μL of Termination Buffer to stop kinase reaction.

Place plate(s) into MSA device.

5. Measure the Product/Sum ratio.
6. Calculate Inhibition % of compound as follows;

Inhibition (%)＝(1－(C－A)/(B－A)) x 100

, where A equals negative control, B equals positive control and C equals test sample Product/Sum ratio.

Separation conditions for 12-Sipper chip

Parameter	Setting
Chip	12-Sipper Chip
Analysis Results Type	Off-Chip Mobility Shift
Threshold	None
Baseline Threshold	None
Peak Order	Product First
Downstream (V)	- 600
Upstream (V)	- 1800
Base Pressure (psi)	- 0.1 (TC372) / - 0.5 (TF570) Note: Please adjust base pressure (psi) to the chip type you apply.
Pressure (psi)	- 1.1
Initial Delay (sec)	0
End of Plate Delay (sec)	70
Final Delay (sec)	17
Sample sip Time (sec)	0.2
Post Sample Buffer sip Time (sec)	35
Dye sip Time (sec)	0.2
Post Dye sip Time (sec)	45

Detection System

Light Source	Blue Laser
Excitation	488 nm
Emission	510 - 550 nm

The typical separation pattern of substrate and product with KINASE MSA Kit is shown below.

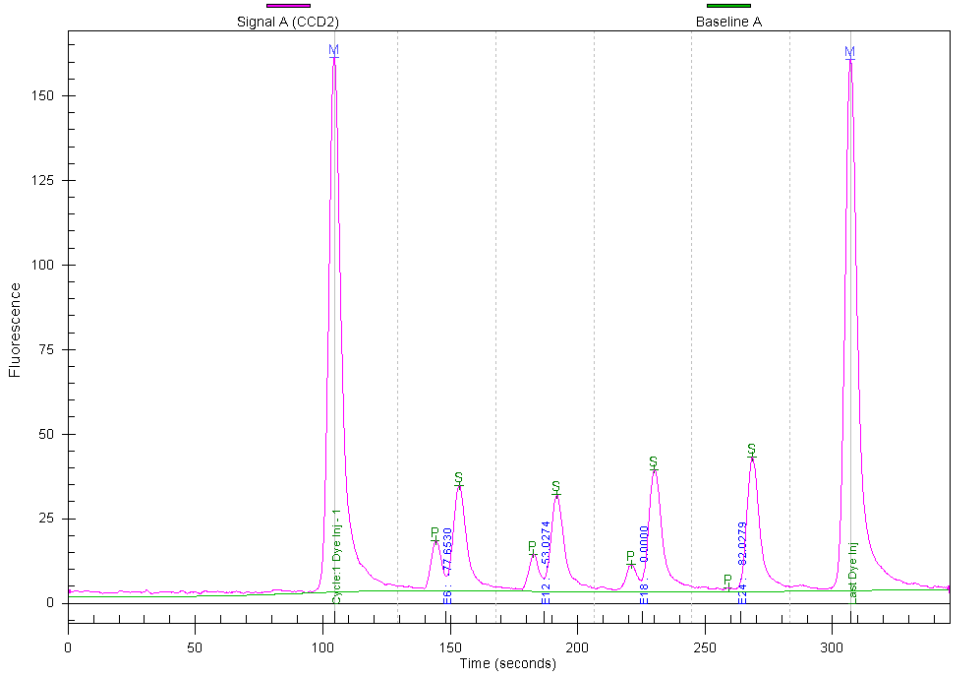


Fig.1 Typical separation pattern under the described separation conditions on page 4.

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