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 (MSA). This kit includes Assay Buffer, Termination Buffer, human protein kinase, ATP/Substrate/Metal and a protocol to perform 384 well plate assay.

Components (400dpt x 1set)

Materials	Volume	Storage
10 x Assay Buffer	5 mL x 1	-80°C
KINASE *	25 µL x 1	-80°C
5 x ATP/substrate/metal	0.6 mL x 1	-80°C (light shielding)
4 x Termination Buffer	7.5 mL x 1	-80°C

* Concentration of the kinase; 100 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 4 mL. Dilute with 36 mL of distilled water (total volume: 40 mL). 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 distilled water (total volume: 2.25 mL). Bring the solution to room temperature and shield from light until use.

Enzyme Solution

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

Termination Buffer

Thaw 4 x Termination Buffer and dilute 7 mL into 21 mL of distilled water (total volume: 28 mL). Bring Termination Buffer to room temperature (25±2℃) prior to 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.

Coating-3 Reagent (CR-3) 30 mL bottle from PerkinElmer Inc., cat. no. 760050

Separation Buffer

100 mM HEPES (pH7.5), 10 mM $MgCl_2$, 0.01 % Triton X-100, 10 mM EDTA-2Na,
1 % DMSO, 0.1 % Coating-3 Reagent.

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

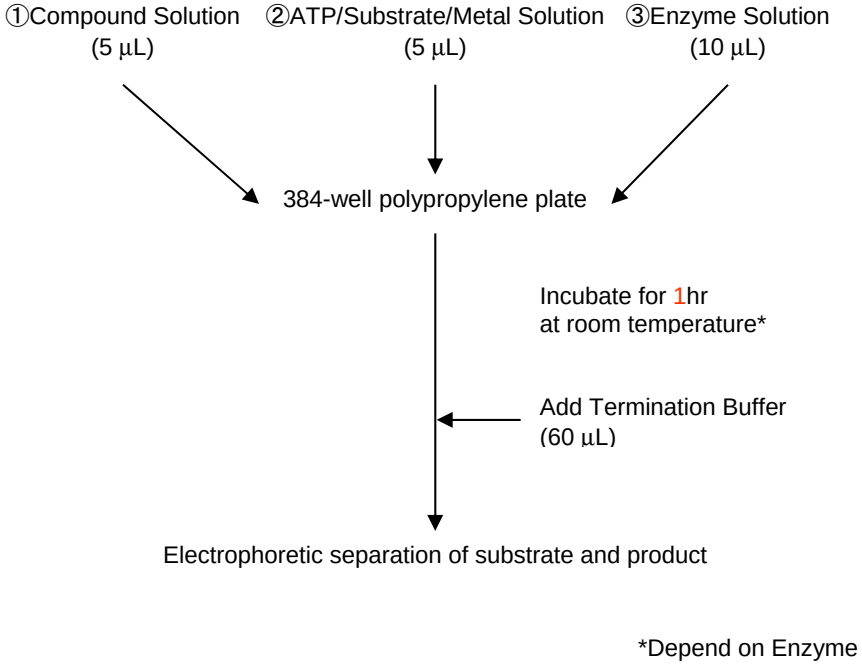
20 mM HEPES (pH7.5), 0.01 % Triton X-100, 2 mM DTT
1000 nM Substrate, 10 µM ATP, 5 mM Metal

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 well 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 Termination Buffer to stop kinase reaction.
   Place plate(s) into MSA device.
5. Measure the Product/Sum ratio.

Illustration of Assay Procedures:



*Depend on Enzyme

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 (760404) / - 0.5 (760394) 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.

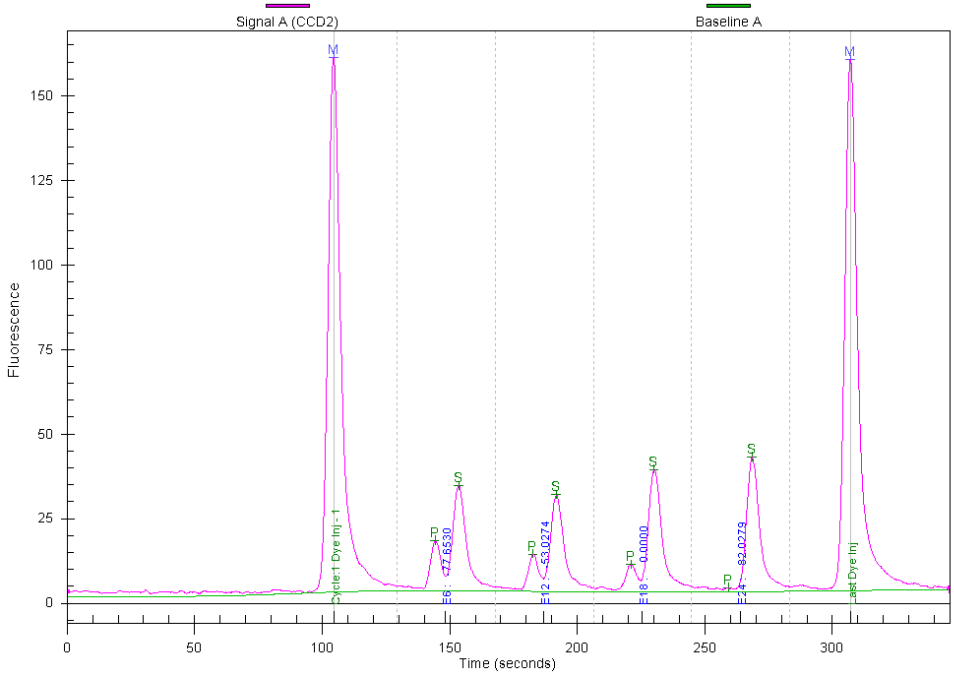


Fig.1 Typical separation pattern under the described separation conditions on page 4.

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