Assay procedure may vary depending on the kinase to test.
Please inquire for the details.

QS S Assist STK_ELISA Kit

Description

STK ELISA Kit is designed for use in pharmacological assays for STK that detects phosphorylated serine (or threonine) with anti-phosphoserine/threonine antibody and horseradish peroxidase (HRP)-conjugated anti-Ig antibody. The kit includes assay buffer, human protein kinase, ATP/Substrate/Metal, anti-phosphoserine/threonine antibody, HRP-conjugated anti-Ig antibody and a protocol to perform assay.

Components (500 dpt)

Materials	Volume	Storage
10 x Assay Buffer	10 mL	-80°C
500 x STK *	30 µL	-80°C
5 x ATP/Substrate/Metal	1400 µL	-80°C
1000 x 1st Anti-phospho-S/T Antibody	70 µL	-20°C
2000 x HRP-2nd anti-Ig Antibody	35 µL	4°C

* Concentration of the kinase; 100 µg/mL

Please avoid repeated freeze-thaw cycles.

Materials provided (for 1 plate)

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

Assay Buffer

Thaw 10 x Assay Buffer on ice. For one plate (96 wells) determination, dilute 1.5 mL of 10 x Assay Buffer with 13.5 mL of distilled water and not stored. The Assay Buffer is kept at room temperature before use.

ATP/Substrate/Metal Solution

Thaw 5 x ATP/Substrate/Metal on ice. For one plate (96 wells) determination, dilute 250 µL of 5 x ATP/Substrate/Metal with 1 mL of Assay Buffer. ATP/Substrate/Metal Solution is kept on ice before use.

Enzyme Solution

Thaw 500 x STK on ice. For one plate (96 wells) determination, dilute 5 µL of enzyme with 2.495 mL of Assay Buffer. Keep the diluted enzyme solution on ice before use.

1st Antibody Solution

For one plate (96 wells) determination, dilute 12 µL of Anti-phospho-1st Antibody with 12 mL of Blocking Buffer (1,000-fold dilution). This 1st antibody solution is kept at room temperature before use.

2nd Antibody Solution

For one plate (96 wells) determination, dilute 6 µL of HRP-conjugated anti-Ig antibody with 12 mL of Blocking Buffer (2,000-fold dilution). The 2nd antibody solution is kept at room temperature before use

Choice of plate type depends on the kinase to test.
Please inquire for the details.

Materials required

Streptavidin-coated 96-well plate (Nunc #436014 / PerkinElmer #4009-0010)

Compound Solution

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

Stop Solution

40 mM EDTA sodium salt (adjusted to pH7.5).

Wash Buffer

50 mM Tris-HCl buffer (pH 7.5) containing 150 mM NaCl and 0.02% Tween 20. Wash Buffer is kept at room temperature before use.

Blocking Buffer

Wash Buffer containing 0.1% BSA. Blocking Buffer is kept at room temperature before use.

Color Reagent

TMB Peroxidase substrate elisa (TMBE-100) (Moss, Inc., MA. USA.).

0.1 M H_2SO_4

Summary of Reagent Preparation

Reagent	Preparation （for 1 plate）
Assay Buffer	10 x Assay Buffer, 1.5 mL + distilled water, 13.5 mL
Enzyme Solution	500 x STK, 5 µL + Assay Buffer, 2.495 mL
ATP/Substrate/Metal Solution	5 x ATP/Substrate/Metal, 250 µL + Assay Buffer, 1 mL
1st Antibody Solution	1000 x 1st-Antibody, 12 µL + Blocking Buffer, 12 mL
HRP-2nd Antibody Solution	2000 x HRP-2nd-Antibody, 6 µL + Blocking Buffer, 12 mL

Example of Reaction

Sample	Compound Solution (µL)	Vehicle (µL)	ATP/Substrate/Metal Solution (µL)	Enzyme Solution (µL)	Assay Buffer (µL)
A	－	10	10	－	20
B	－	10	10	20	－
C	10	－	10	20	－

Calculate of inhibition percentage of compound as follows; Inhibition (%)＝(1－(C－A)/(B－A)) x 100

Final Concentration of Components in Reaction Mixture

15 mM Tris-HCl(pH7.5), 0.01% Tween 20, 2 mM DTT

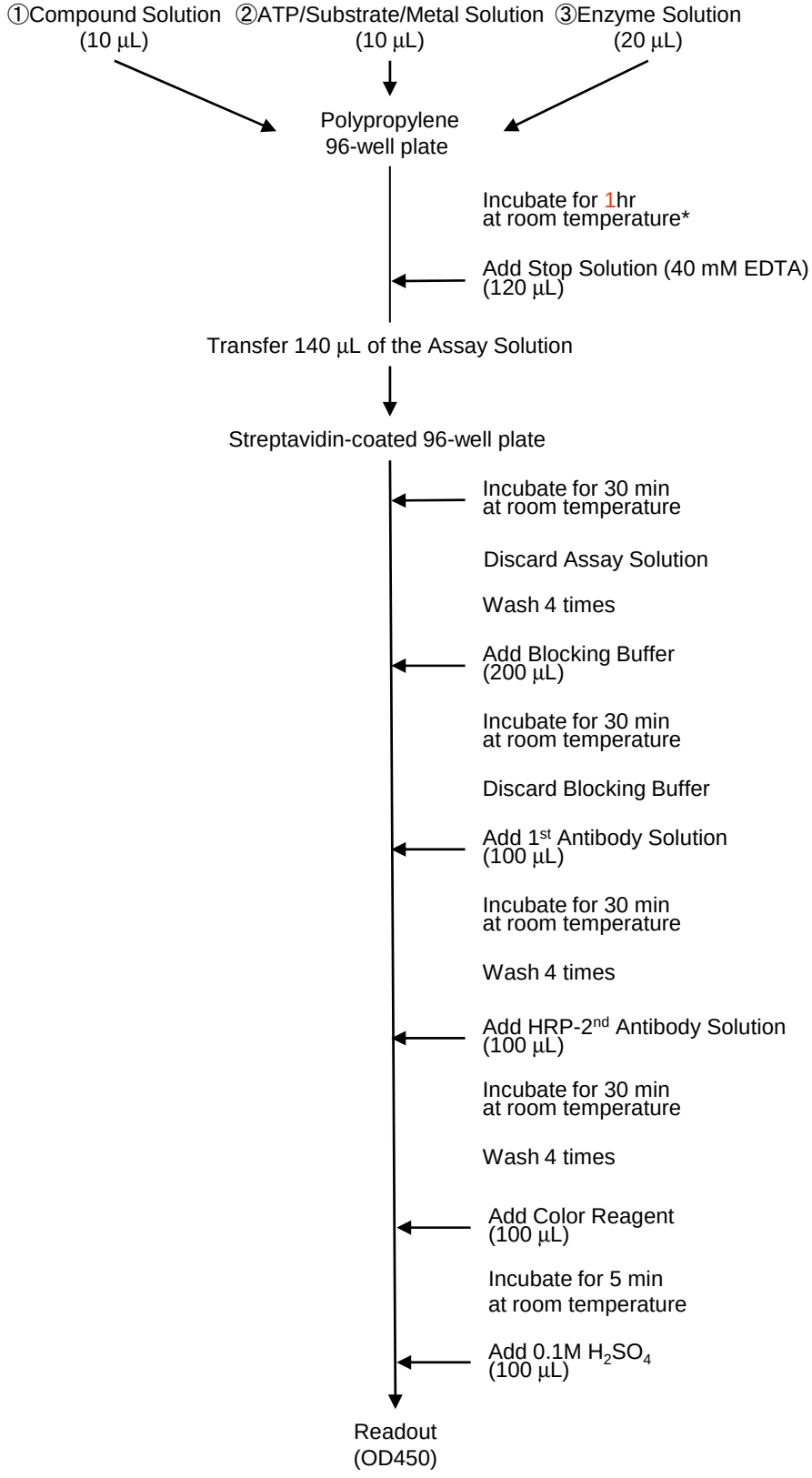
180 nM Substrate, 5 µM ATP, 10 mM Mg

ASSAY PROCEDURE:

All procedures are performed at room temperature.

1. Add 10 µL of Vehicle (4% DMSO) to wells of "A" and "B" and Compound Solution to well of "C" of a polypropylene 96-well plate.
2. Add 10 µL of ATP/Substrate/Metal Solution to each well.
3. Add 20 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 120 µL of Stop Solution to each well in order to stop the kinase reaction.
5. Transfer 140 µL of the Assay Solution of each well to the Streptavidin-coated 96-well plate. Incubate for 30 minutes.
6. Discard each well solution and wash with Wash Buffer, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (250 µL) using a multi-channel pipette. Complete removal of liquid at each step is essential for good performance. After the last wash remove any remaining solutions completely by aspirating or inverting plate and blotting it against clean paper towels.
7. Add 200 µL of Blocking Buffer to each well in order to block plates. Cover the plate and incubate for 30 minutes.
8. Discard Blocking Buffer by inverting the plate and blotting against clean paper towels to remove remaining solutions completely.
9. Add 100 µL of 1st Antibody Solution. Cover the plate and incubate for 30 minutes.
10. Repeat the discard/wash as in step 6.
11. Add 100 µL of HRP-2nd Antibody Solution to each well. Cover the plate and incubate for 30 minutes.
12. Repeat the discard /wash as in step 6.
13. Add 100 µL of Color Reagent to each well. Incubate for 5 minutes. Avoid placing the plate in direct light.
14. Add 100 µL of 0.1 M H_2SO_4 to each well. Gently tap the plate to ensure thorough mixing.
15. Measure the absorbance of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 or 570 nm. If wavelength correction is not available, subtract readings at 540 or 570 nm. This subtraction will correct for optical imperfections in the plate. Direct readings at 450 nm without correction may be higher or less accurate.
16. Calculate of inhibition percentage of compound as follows; Inhibition (%)＝(1－(C－A)/(B－A)) x 100

Illustration of assay procedures



*Depend on Enzyme

Assay result example

The inhibitory effect of Reference compound on STK evaluated using STK_ELISA Kit is shown below.

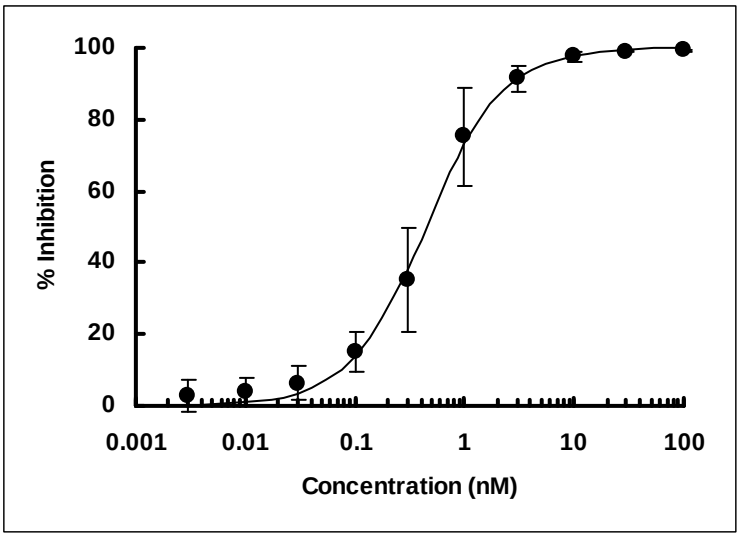


Figure.1. Reference compound inhibition curve.