# 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 |
|---|---|---|
| x 10 Assay Buffer | 10 mL | -80°C |
| x 500 STK | 30 µL | -80°C |
| x 5 ATP/Substrate/Metal | 1500 µL | -80°C |
| x 1000 1st Anti-phospho-S/T Antibody | 80 µL | -20°C |
| x 2000 HRP-2nd anti-Ig Antibody | 40 µL | 4°C |

Please avoid repeated freeze-thaw cycles.

## Materials provided

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

### Assay Buffer

Thaw Assay Buffer (x 10) on ice. For one plate (96 wells) determination, dilute 1.5 mL of Assay Buffer (x 10) 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 ATP/Substrate/Metal (x 5) on ice. For one plate (96 wells) determination, dilute 250 µL of ATP/Substrate/Metal (x 5) with 1 mL of Assay Buffer. ATP/Substrate/Metal solution is kept at room temperature.

### Enzyme Solution

Thaw STK (x 500) 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

## Materials required

**Glutathione/Streptavidin-coated 96-well plate**

Nunc #436032/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-1000) (Moss, Inc., MA. USA.).

**0.1 M $H_2SO_4$**

**Summary of Reagent Preparation**

| Reagent | Preparation |
|---|---|
| Assay Buffer | Assay Buffer (x 10), 1.5 mL + distilled water, 13.5 mL |
| Enzyme | STK (x 500), 5 µL + Assay Buffer, 2.495 mL |
| ATP/Substrate/Metal | ATP/Substrate/Metal (x 5), 250 µL + Assay Buffer, 1 mL |
| 1st Antibody Solution | 1st-Antibody (x 1,000), 12 µL + Blocking Buffer, 12 mL |
| HRP-2nd Antibody Solution | HRP-2nd-Antibody (x 2,000), 6 µL + Blocking Buffer, 12 mL |

**Example of Reaction**

| Sample | Compound solution (µL) | Vehicle (µL) | ATP/Substrate/Metal (µL) | Enzyme (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

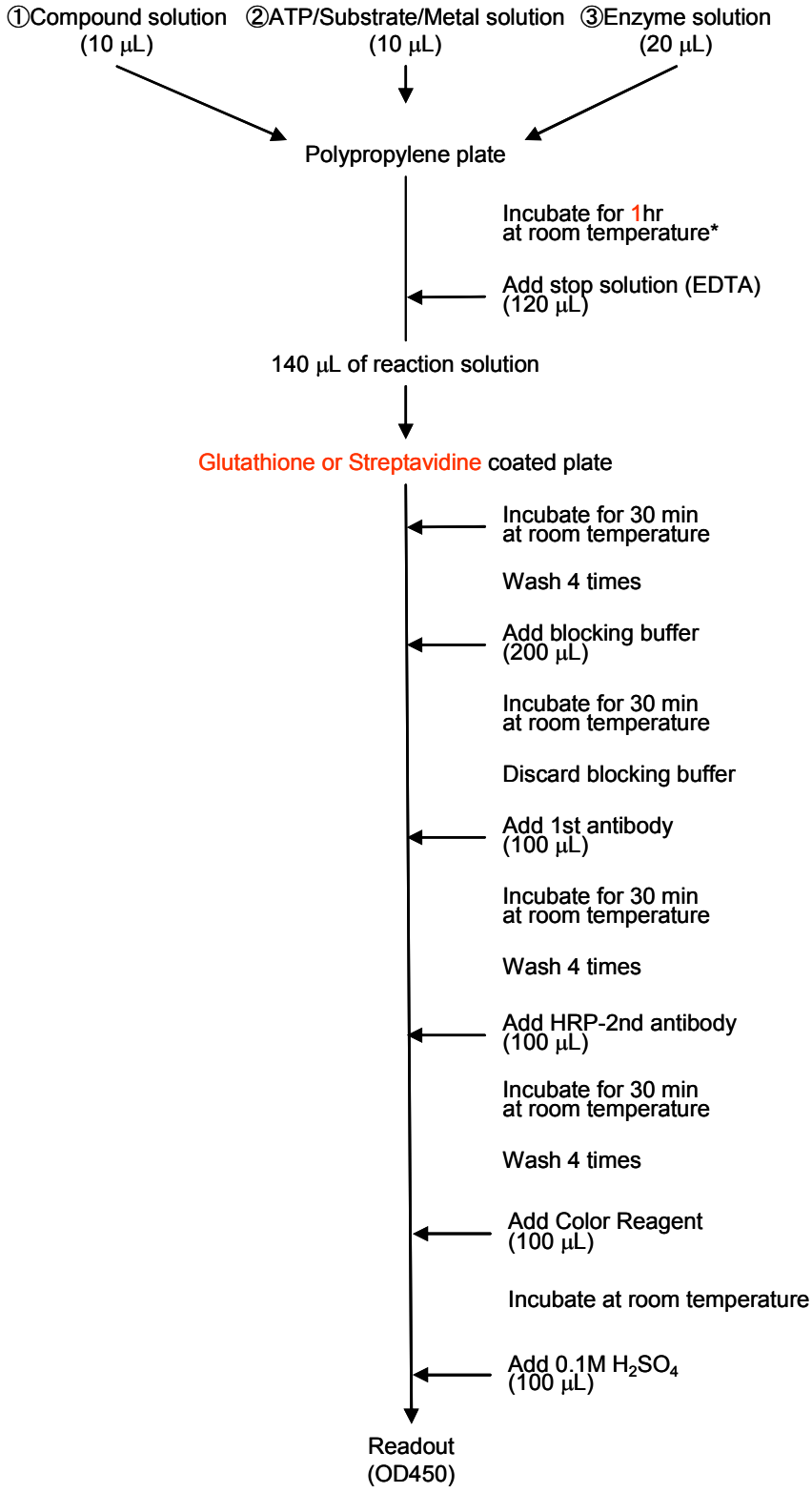
180 nM substrate, 5 µM ATP, 10 mM Mg

**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 Glutathione/Streptavidin-coated 96-well plate. Incubate for 30 minutes.
6. Aspirate each well 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 1$^{st}$ Antibody Solution. Cover the plate and incubate for 30 minutes.
10. Repeat the aspiration/wash as in step 6.
11. Add 100 µL of HRP-2$^{nd}$ Antibody Solution to each well. Cover the plate and incubate for 30 minutes.
12. Repeat the aspiration/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



**Assay result example**

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