QS S Assist TK_ELISA Kit

Description

TK ELISA Kit is designed for use in pharmacological assays for TK that detects phosphorylated substrate with horseradish peroxidase (HRP)-conjugated anti-phosphotyrosine antibody. The kit includes assay buffer, human protein kinase, ATP/Substrate/Metal and a protocol to perform 96 well plate assays.

Components (500 dpt)

Materials	Volume	Storage
10 x Assay Buffer	10 mL	-80°C
500 x TK	40 µL	-80°C
5 x ATP/Substrate/Metal	1500 µL	-80°C

Please avoid repeated freeze-thaw cycles.

Materials provided

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

Assay Buffer

Thaw Assay Buffer (10 x) on ice. For one plate (96 wells) determination, dilute 1.5 mL of Assay Buffer (10 x) with 13.5 mL of distilled water and not stored. The Assay Buffer is kept at 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 ATP/Substrate/Metal (5 x) on ice. For one plate (96 wells) determination, dilute 250 µL of ATP/Substrate/Metal (5 x) with 1 mL of Assay Buffer. ATP/Substrate/Metal solution is kept at room temperature.

Enzyme Solution

Thaw TK (500 x) and 500-fold dilute it with Assay Buffer. Please keep the enzyme on ice before use.

Materials required

Streptavidin-coated 96-well plate

Delfia® Streptavidin Microtitration Strips (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.

Wash Buffer

50 mM Tris-HCl buffer (pH 7.5±0.5) containing 150 mM NaCl and 0.02% Tween 20.

Thaw Wash Buffer (x 10). Dilute 25 mL of Wash Buffer (x 10) with 225 mL of distilled Water. Wash Buffer is kept at room temperature before use.

Blocking Buffer

Wash Buffer containing 0.1% BSA.

Thaw 10 % BSA (x 100) on ice. For one plate (96 wells) determination, dilute 400 μL of BSA (x 100) with 39.6 mL of Wash Buffer. Blocking Buffer is kept at room temperature before use.

HRP-conjugated anti-pY antibody

PY20 (Santa Cruz Biotechnology Inc.) sc-508 HRP

Dilute PY20 with Blocking Buffer. For one plate (96 wells) determination, 10 mL of diluted PY20 is required. Optimal dilution should be determined by the investigator (in case of Lot. I1007, 400-fold dilution is recommended)

Color Reagent

TMB Peroxidase substrate elisa (TMBE-1000) (Moss, Inc.)

0.1 M H_2SO_4

Summary of Reagent Preparation

Reagent	Preparation for one plate (96-well)
Assay Buffer	Assay Buffer (10 x), 1.5 mL + distilled water, 13.5 mL
Kinase	TK (500 x) 5 µL + Assay Buffer, 2.495 mL
ATP/Substrate/Metal	ATP/Substrate/Metal (5 x), 250 µL + Assay Buffer, 1 mL
Anti-pY Antibody	HRP-Antibody (400 x) , 30 µL + Blocking Buffer, 12 mL

Final Concentration of Components in Reaction Mixture

15 mM Tris-HCl(pH7.5±0.5), 0.01% Tween 20, 2 mM DTT

250 nM substrate, 20 µM ATP, 5 mM Mg

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

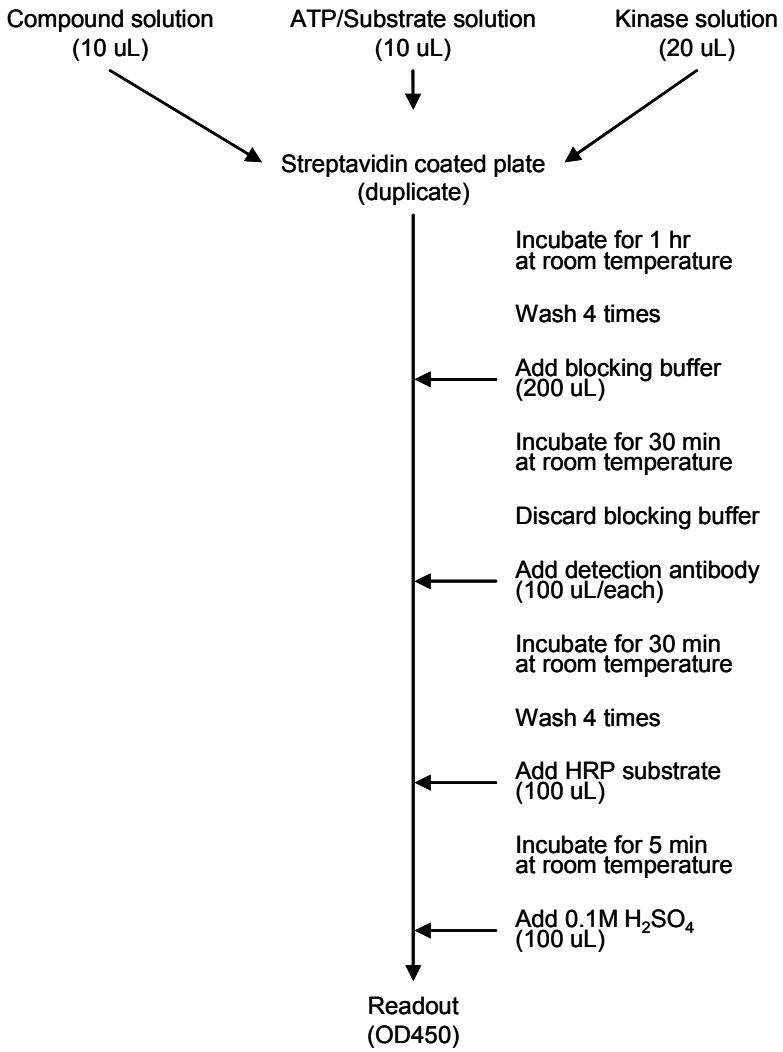
ASSAY PROCEDURE:

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”.
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. 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.
5. Add 200 µL of Blocking Buffer to each well in order to block plates. Cover the plate and incubate for 30 minutes.

6. Discard Blocking Buffer by inverting the plate and blotting against clean paper towels to remove remaining solutions completely.
7. Add 100 μL of diluted HRP-conjugated anti-pY antibody to each well. Cover the plate and incubate the plate for 30 minutes.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Color Reagent to each well. Incubate for 5 minutes. Avoid placing the plate in direct light.
10. Add 100 μL of 0.1 M H_2SO_4 to each well. Gently tap the plate to ensure thorough mixing.
11. Measure the absorbance of each well immediately, using a microplate reader set to 450nm. 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.
12. 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 Staurosporine on TK evaluated using TK ELISA Kit is shown below.