Store at -80°C

#7630

HTScan® TSK2 Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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Products Included	Products #	Kit Quantity
Phospho-PLK (Ser137) Antibody	5070	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
PLK (Ser137) Biotinylated Peptide	1300	1.25 ml
TSK2 Kinase (recombinant, human)	7629	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human TSK2 kinase. It includes active TSK2 kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-serine/threonine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: RRS*LL

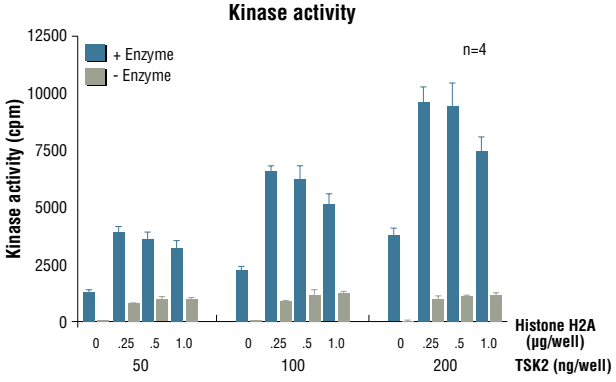
Molecular Weights: Peptide substrate, Biotin-peptide: 1,646 Daltons. GST-TSK2 Kinase domain: 74 kDa.

Background: Testis-specific kinases (TSKs) are serine/threonine kinases that may play a role in sperm function. Three TSKs, TSK1, TSK2 and TSK3 have been identified. Open reading frames (ORF) within the TSK gene code for the 3 isoforms: 367 amino acids for TSK1, 358 amino acids for TSK2 and 275 amino acids for TSK3. The kinase domains are located near the NH2-terminal regions of the TSK proteins and contain conserved amino acid residues characteristic of a serine/threonine kinase. While TSK1 and TSK2 both have a 100 amino acid domain located C-terminally to the kinase domain, TSK3 is composed essentially of a serine/threonine kinase domain. TSK1 and TSK2 messenger RNA is expressed exclusively in the testis of mature males. An in vitro kinase assay of immunoprecipitates containing either TSK1 or TSK2 revealed serine phosphorylation of a coprecipitating protein of approximately 65 kDa. This substrate protein is also expressed exclusively in the testis as demonstrated by northern and dot blot analyses (1,2). While TSK1 and TSK2 form detergent resistant complexes during immunoprecipitation, TSK3 does not associate with either protein. Expression of TSK3 is induced at puberty, is restricted to the interstitial Leydig cells of postpubertal males, persists throughout adulthood and is implicated in regulating androgenesis and/or Leydig cell proliferation (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human TSK2 (Met1-Thr358) (GenBank Accession No. NM_053006) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The substrate peptide was selected using our Serine/Threonine Kinase Substrate Screening Kit #7400. Phospho-PLK (Ser137) antibody #5070 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified TSK2 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the TSK2 kinase was determined using a radiometric filter binding assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose dependency [Fig.4] assays were performed to verify TSK2 activity using the TSK2 substrate peptide provided in this kit. TSK2 sensitivity to the inhibitor staurosporine was measured using the TSK2 substrate peptide provided in this kit [Fig.5].



◀ *Figure 1. TSK2 kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 1 µM ATP, 2.5 µg/50 µl PEG20,000, Substrate: Histone H2A, variable, and recombinant TSK2: variable.*

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:
Serine/Threonine Kinase Substrate Screening Kit #7400

TSK2 Kinase #7629

Phospho-PLK (Ser137) Antibody #5070

PLK (Ser137) Biotinylated Peptide #1300

Staurosporine #9953

Background References:

(1) Bielke, W. et al. (1994) *Gene* 139, 235–239.

(2) Hao, Z. et al. (2004) *Mol Hum Reprod* 10, 433–444.

(3) Kueng, P. et al. (1997) *J Cell Biol* 139, 1851–1859.

(4) Zuercher, G. et al. (2000) *Mech Dev* 93, 175–177.

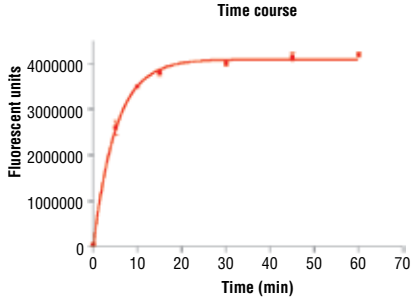


Figure 2. Time course of TSK2 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of TSK2 substrate peptide (#1300) by TSK2 kinase. In a 50 µl reaction, 50 ng TSK2 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

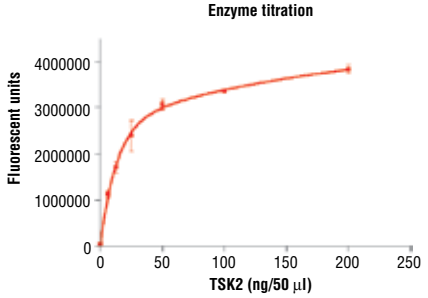


Figure 3. Dose dependence curve of TSK2 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide (#1300) by TSK2 kinase. In a 50 µl reaction, increasing amounts of TSK2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

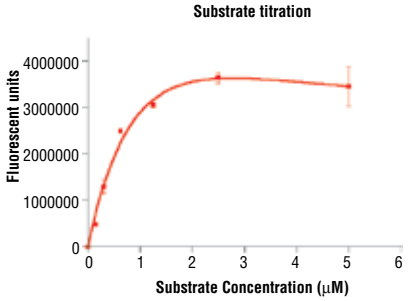


Figure 4. Peptide concentration dependence of TSK2 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide (#1300) by TSK2 kinase. In a 50 µl reaction, 50 ng of TSK2 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

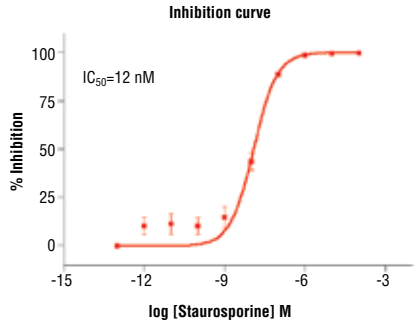


Figure 5. Staurosporine inhibition of TSK2 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of TSK2 substrate peptide (#1300) by TSK2 kinase. In a 50 µl reaction, 50 ng TSK2, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® TSK2 Kinase Assay Kit

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [1 ml 10X Kinase Buffer 250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Transfer 1.2 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 4 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng TSK2 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody, Phospho-PLK (Ser137) Antibody, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
   - **a.** Excitation Filter: 340 nm
   - **b.** Emission Filter: 615 nm
   - **c.** Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

***NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com