

# HTScan® SGK3 Kinase Assay Kit

✓ 100 assays  
(96 Well Format)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

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
SGK3 Kinase (recombinant, human)	7620	5 µg

**Description:** The kit provides a means of performing kinase activity assays with recombinant full-length human SGK3 kinase. It includes active SGK3 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-PLK (Ser137): 1,945 Daltons. GST-SGK3 Kinase: 83 kDa.

**Background:** Serum and glucocorticoid-inducible kinase (SGK), a serine/threonine kinase, is a close relative of Akt (1). SGK is rapidly induced in response to a variety of stimuli, including serum, glucocorticoid, follicle stimulating hormone, osmotic shock and mineralocorticoids. SGK activation can be accomplished via HGF PI3K-dependent pathways and by integrin-mediated PI3K-independent pathways (2,3). Induction and activation of SGK has been implicated in activating the modulation of antiapoptotic and cell cycle regulation (4-6). SGK also plays an important role in activating certain potassium, sodium and chloride channels, suggesting its involvement in the regulation of processes such as cell survival, neuronal excitability and renal sodium excretion (2). SGK is negatively regulated by ubiquitin modification and proteasome degradation (7).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full-length human SGK3 (Met1-Leu496) (GenBank Accession No. NM\_013257.3) 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 SGK3 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the SGK3 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 SGK3 activity using the SGK3 substrate peptide provided in this kit. SGK3 sensitivity to the inhibitor staurosporine was measured using the SGK3 substrate peptide provided in this kit [Fig.5].

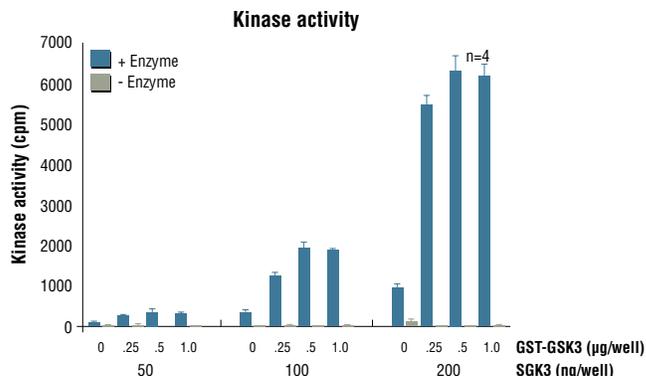


Figure 1. SGK3 kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl<sub>2</sub>, 3 mM MnCl<sub>2</sub>, 3 µM Na-orthovanadate, 1.2 mM DTT, 1 µM ATP, 2.5 µg/50 µl PEG20,000. Substrate: GST-GSK3 (14-27), variable and recombinant SGK3, 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

Phospho-PLK (Ser137) Antibody #5070

PLK (Ser137) Biotinylated Peptide #1300

SGK3 Kinase #7620

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

#### Background References:

- (1) Webster, M.K. et al. (1993) *Mol. Cell. Biol.* 13, 2031–2040.
- (2) Kobayashi, T. and Cohen, P. (1999) *Biochem. J.* 339, 319–328.
- (3) Park, J. et al. (1999) *EMBO J.* 18, 3024–3033.
- (4) Brunet, A. et al. (2001) *Mol. Cell. Biol.* 21, 952–965.
- (5) Mikosz, C.A. et al. (2001) *J. Biol. Chem.* 276, 16649–16654.
- (6) Hayashi, M. et al. (2001) *J. Biol. Chem.* 276, 8631–8634.
- (7) Brickley, D.R. et al. (2002) *J. Biol. Chem.* 277, 43064–43070.

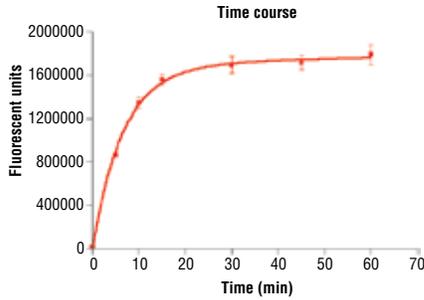


Figure 2. Time course of SGK3 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of SGK3 substrate peptide (#1300) by SGK3 kinase. In a 50 µl reaction, 50 ng SGK3 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

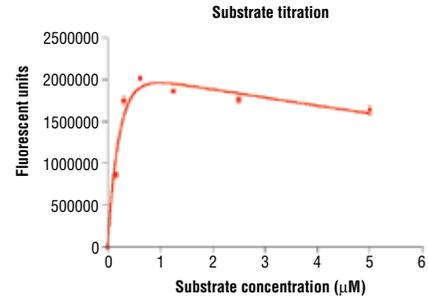


Figure 4. Peptide concentration dependence of SGK3 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide (#1300) by SGK3 kinase. In a 50 µl reaction, 50 ng of SGK3 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

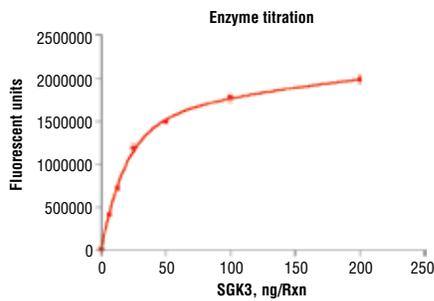


Figure 3. Dose dependence curve of SGK3 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of substrate peptide (#1300) by SGK3 kinase. In a 50 µl reaction, increasing amounts of SGK3 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

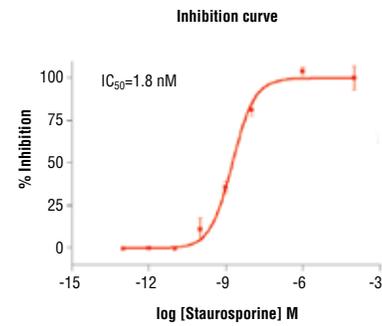


Figure 5. Staurosporine inhibition of SGK3 kinase activity: DELFIA® data generated using Phospho-PLK (Ser137) Antibody #5070 to detect phosphorylation of SGK3 substrate peptide (#1300) by SGK3 kinase. In a 50 µl reaction, 50 ng SGK3, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

## Protocol for HTScan® SGK3 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

*DELFLIA® is a registered trademark of PerkinElmer Life Sciences*

### 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<sub>2</sub>O 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<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH<sub>2</sub>O 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<sub>2</sub>  
5 mM β-glycerophosphate  
0.1 mM Na<sub>3</sub>VO<sub>4</sub>  
2 mM DTT  
200 µM ATP  
1.5 µM peptide  
50 ng SGK3 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<sub>2</sub>O/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 DELFLIA® or Colorimetric ELISA detection methods please use the following protocols.

### DELFLIA® 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 DELFLIA® 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 DELFLIA®

DELFLIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)  
DELFLIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)  
DELFLIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)  
DELFLIA® 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