Store at -80°C

#7660

HTScan® MAPKAPK-5 Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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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-Tyrosine Hydroxylase (Ser40) Antibody	2791	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
Tyrosine Hydroxylase (Ser40) Biotinylated Peptide	1132	1.25 ml
MAPKAPK-5 Kinase (recombinant, human)	7659	2 x 5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human MAPKAPK-5 kinase. It includes active MAPKAPK-5 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: RRQS*LIE

Molecular Weights: Peptide substrate, Biotin- Tyrosine Hydroxylase (Ser40): 2,326 Daltons. GST-MAPKAPK-5 Kinase: 84 kDa.

Background: MAPKAPK5 belongs to the mitogen-activated protein kinase (MAPK) activated protein kinases (MK) subfamily that includes MAPKAPK2/MK2 and MK3/3pK. The MK subfamily is part of a family of protein kinase subfamilies downstream of MAPK pathways and includes ribosomal S6 kinase (RSKs), mitogen and stress activated kinases (MSKs) and MAPK-interacting kinases (MNKs). All MKs are activated by MAPK pathways and mediate important processes such as gene expression, cell-cycle progression and have been implicated in inflammation and cancer (1,2). MAPKAPK5 shows binding to and activation by the p38 kinase and extracellular-regulated kinases (ERK) (3,4). Recently, MAPKAPK5 was shown to be activated by ERK3 and act as a chaperone to ERK3 (5,6). While overexpressed MAPKAPK5 shares similar substrates with MAPKAPK2, such as HSP27 and glycogen synthase, recent work with MAPKAPK5 knock-out mice indicate distinct substrates and functional properties (7).

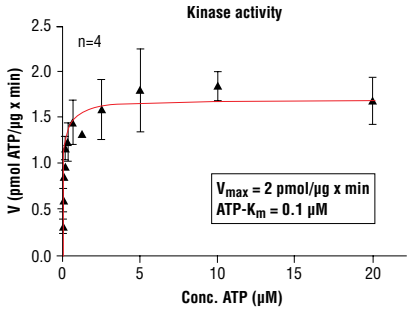

Figure 1. MAPKAPK-5 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP, variable, 2.5 µg/50 µl PEG20.000, Substrate: Tetra (LRRWSLG), 3 µg/50 µl and recombinant MAPKAPK-5: 200 ng/50 µl.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human MAPKAPK-5 (Met1-Gln473) (GenBank Accession No. NM_139078) 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-Tyrosine Hydroxylase (Ser40) Antibody #2791 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

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

Background References:

(1) Roux, P.P. and Blenis, J. (2004) *Microbiol. Mol. Biol. Rev.* 68, 320–344.

(2) Gaestel, M. (2006) *Nat. Rev. Mol. Cell Biol.* 7, 120–130.

(3) New, L. et al. (1998) *EMBO J.* 17, 3372–3384.

(4) Ni, H. et al. (1998) *Biochem. Biophys. Res. Commun.* 243, 492–496.

(5) Schumacher, S. et al. (2004) *EMBO J.* 23, 4770–4779.

(6) Seternes, O.M. et al. (2004) *EMBO J.* 23, 4780–4791.

(7) Shi, Y. et al. (2003) *Mol. Cell Biol.* 23, 7732–7741.

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

MAPKAPK-5 Kinase #7659

Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791

Tyrosine Hydroxylase (Ser40) Biotinylated Peptide #1132

Staurosporine #9953

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

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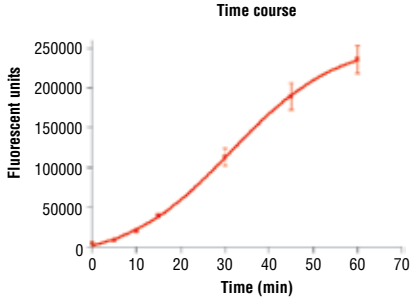

Figure 2. Time course of MAPKAPK-5 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of MAPKAPK-5 substrate peptide #1132 by MAPKAPK-5 kinase. In a 50 µl reaction, 100 ng MAPKAPK-5 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

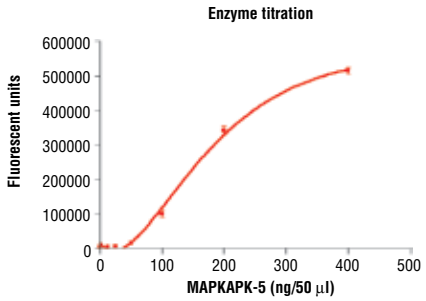

Figure 3. Dose dependence curve of MAPKAPK-5 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of substrate peptide #1132 by MAPKAPK-5 kinase. In a 50 µl reaction, increasing amounts of MAPKAPK-5 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

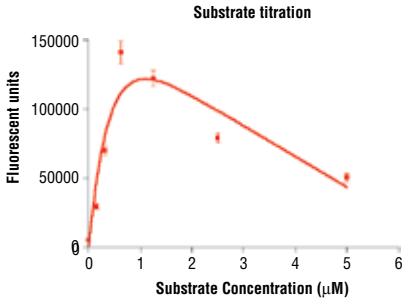

Figure 4. Peptide concentration dependence of MAPKAPK-5 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of substrate peptide #1132 by MAPKAPK-5 kinase. In a 50 µl reaction, 100 ng of MAPKAPK-5 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

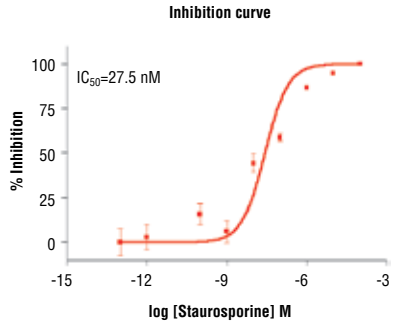

Figure 5. Staurosporine inhibition of MAPKAPK-5 kinase activity: DELFIA® data generated using Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791 to detect phosphorylation of MAPKAPK-5 substrate peptide #1132 by MAPKAPK-5 kinase. In a 50 µl reaction, 100 ng MAPKAPK-5, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

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Protocol for HTScan® MAPKAPK-5 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 0.6 ml of 4X Reaction buffer to each enzyme tube to make 4X reaction cocktail ([enzyme]) = 8 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
100 ng MAPKAPK-5 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-Tyrosine Hydroxylase (Ser40) 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

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