#7556 Store at –80°C

HTScan™ IKKε Kinase Assay Kit

☑ 100 Assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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new 10/05

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

Products Included	Product #	Kit Quantity
Phospho-(Ser/Thr) Phe Antibody	9631	30 µl
Kinase Buffer (10X)	9802	15 ml
ATP (10 mM)	9804	1 ml
PAK1 (Ser144)/ PAK2 (Ser141) Biotinylated Peptide	1134	1.25 ml
IKKε Kinase	7553	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human IKKε kinase. It includes active IKKε 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: YMS*FT

Molecular Weights: Peptide substrate, Biotin-PAK1 (Ser144)/PAK2 (Ser141) peptide: 1,775 Daltons. GST-IKKε Kinase: 110 kDa

Background: The NFκB/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory IκB proteins (1-3). Most agents that activate NFκB do so through a common pathway based on phosphorylation-induced, proteasome-mediated degradation of IκB (3-7). The key regulatory step in this pathway involves activation of a high molecular weight IκB kinase (IKK) complex, whose catalysis is generally carried out by three tightly associated IKK subunits. IKKα and IKKβ serve as the catalytic subunits of the kinase. IKKγ serves as the regulatory subunit (8-9). Activation of IKK depends on phosphorylation; serines 177 and 181 in the activation loop of IKKβ (176 and 180 in IKKα) are the specific sites whose phosphorylation causes conformational changes resulting in kinase activation (10-13).

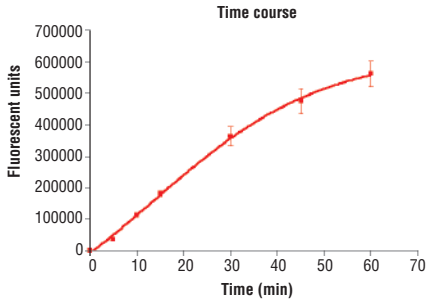

Figure 1. Time course of IKKε kinase activity: DELFIA® data generated using Phospho-(Ser/Thr) Phe Antibody #9631 to detect phosphorylation of IKKε substrate peptide #1134 by IKKε kinase. In a 50 µl reaction, 50 ng IKKε and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Recently, two homologs of IKKα and IKKβ have been described, called IKKε (also known as IKK-i) and TBK-1 (also known as T2K or NAK), and activation of either of these kinases results in NFκB activation. The kinase domain of IKKε is located in its amino-terminus, which shares 30% sequence homology with both IKKα and IKKβ. IKKε is expressed predominantly in immune cells, and may play a special role in the immune response (14-18).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human IKKε (Met1-Val716) (GenBank Accession No. NM_014002) 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-(Ser/Thr) Phe Antibody #9631 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

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

Background References:

(1) Baeuerle, P.A. et al. (1988) *Science* 242, 540–546.
(2) Beg, A.A. et al. (1993) *Genes Dev.* 7, 2064–2070.
(3) Finco, T.S. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 11884–11888.
(4) Brown, K. et al. (1995) *Science* 267, 1485–1488.
(5) Brockman, J.A. et al. (1995) *Mol. Cell. Biol.* 15, 2809–2818.
(6) Traenckner, E.B. et al. (1995) *EMBO J.* 14, 2876–2883.
(7) Chen, Z.J. et al. (1996) *Cell* 84, 853–862.
(8) Zandi, E. et al. (1997) *Cell* 91, 243–252.

Storage: Antibodies are supplied in 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

IKKε Kinase #7553

Phospho-(Ser/Thr) Phe Antibody #9631

PAK1 (Ser144)/ PAK2 (Ser141) Biotinylated Peptide #1134

Staurosporine #9953

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com

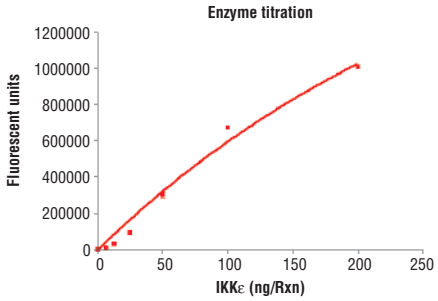

Figure 2. Dose dependence curve of IKKε kinase activity: DELFIA® data generated using Phospho-(Ser/Thr) Phe Antibody #9631 to detect phosphorylation of substrate peptide (#1134) by IKKε kinase. In a 50 µl reaction, increasing amounts of IKKε and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

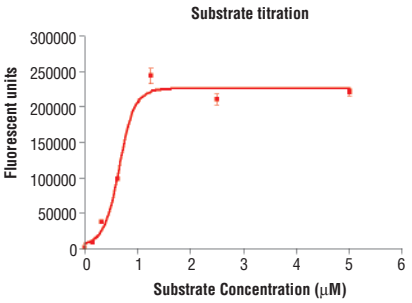

Figure 3. Peptide concentration dependence of IKKε kinase activity: DELFIA® data generated using Phospho-(Ser/Thr) Phe Antibody #9631 to detect phosphorylation of substrate peptide (#1134) by IKKε kinase. In a 50 µl reaction, 50 ng of IKKε and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

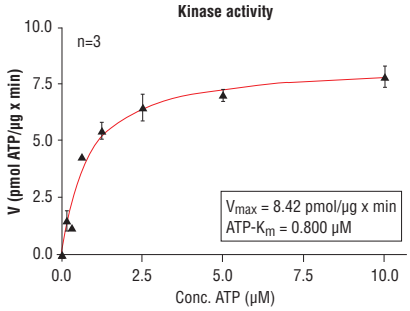

Figure 4. IKKε kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 1 µM ATP, 2.5 µg/50 µl PEG20,000, Substrate: Casein, 10 µg/50 µl, and Recombinant IKKε: 100 ng/50 µl.

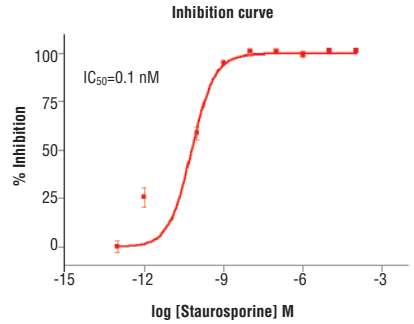

Figure 5. Staurosporine inhibition of IKKε kinase activity: DELFIA® data generated using Phospho-(Ser/Thr) Phe Antibody #9631 to detect phosphorylation of IKKε substrate peptide #1134 by IKKε kinase. In a 50 µl reaction, 50 ng IKKε, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Background References Continued:

(9) Karin, M. et al. (1999) *Oncogene* 18, 6867–6874.
(10) DiDonato, J.A. et al. (1997) *Nature* 388, 548–554.
(11) Mercurio, F. et al. (1997) *Science* 278, 860–866.
(12) Johnson, L.N. et al. (1996) *Cell* 85, 149–158.
(13) Delhase, M. et al. (1999) *Science* 284, 309–313.
(14) Shimada, T. et al. (1999) *Int. Immunol.* 11, 1357–1362.
(15) Peters, R.T. et al. (2000) *Mol. Cell.* 5, 513–522.
(16) Tojima, Y. et al. (2000) *Nature* 404, 778–782.
(17) Bonnard , M. et al. (2000) *EMBO J.* 19, 4976–4985.
(18) Peters, R.T. and Maniats, T. (2001) *Biochim. Biophys. Acta.* 1471, M57–62.

Protocol for HTScan™ IKKε 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.

Additional Solutions and Reagents (Not included)

- Wash Buffer: 1X PBS, 0.05% Tween-20 (PBS/T)
- Bovine Serum Albumin (BSA)
- Stop Buffer: 50 mM EDTA pH 8
- DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
- DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
- DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

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. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 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 (pH7.5)

10 mM $MgCl_2$

5 mM β-glycerophosphate

0.1 mM Na_3VO_4

200 µM ATP

2 mM DTT

1.5 µM peptide

50 ng IKKε 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-(Ser/Thr) Phe Antibody #9631, 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled anti-rabbit antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

***IMPORTANT: 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