Store at -80°C

#7548

IKKβ Kinase

☑5 µg

Cell Signaling TECHNOLOGY®

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REV. 12/06/05

Description: Purified recombinant full length human IKKβ kinase, supplied as a GST fusion protein.

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).

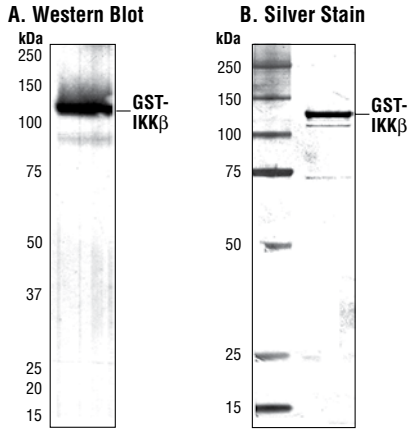

Figure 1. The purity of the GST-IKKβ fusion protein was analyzed using SDS/PAGE followed by anti-IKKβ Western blot (A) or Silver stain (B).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system from a construct containing a full length human IKKβ cDNA kinase domain (Met1-Ser756) (GenBank accession No. AF029684) fragment amino-terminally fused to a GST-HIS_6-Thrombin cleavage site. The protein was then purified by one-step affinity purification using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-IKKβ kinase fusion protien is 87 kDa (apparent molecular weight on SDS PAGE is 120 kDa). The purified kinase fusion protein was quality controlled for purity using SDS-PAGE Silver stain and Western blot [Fig.1]. IKKβ kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure IKKβ activity using HTScan™ IKKβ Kinase Assay Kit #7549 [Fig.3].

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) E*MBO 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.
(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.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ IKKβ Kinase Assay Kit #7549

IκB-α (Ser32) Biotinylated Peptide #1146

Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb #9246

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

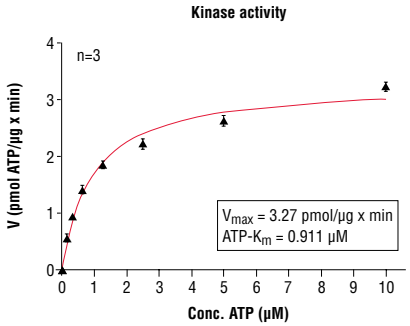

Figure 2. IKKβ kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 uM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: Rb CTF, 1.5 µg/50 µl, Recombinant IKKβ: 50 ng/50 µl.

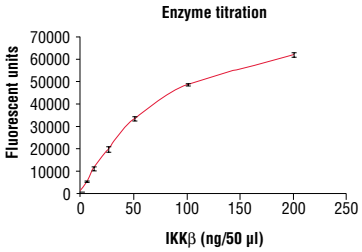

Figure 3. Dose dependence curve of IKKβ kinase activity: DELFIA® data generated using Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb #9246 to detect phosphorylation of substrate peptide (#1146) by IKKβ kinase. In a 50 µl reaction, increasing amounts of IKKβ and 1.5 µM substrate peptide were used per reaction well at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for IKKβ Kinase Assay

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

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
4. Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb #9246
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. IκB-α (Ser32) Biotinylated Peptide #1146
8. DELFIA® Europium-labeled Anti-mouse IgG antibody (PerkinElmer Life Sciences #AD0124)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® 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_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 [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 (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 IKKβ 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 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a IκB-α (Ser32) Biotinylated Peptide and Phospho-IκB-α (Ser32/36) (5A5) Mouse mAb diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:500 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.

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