

IKK β , Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # 103-18G

Lot # U1536-6

Product Description

Full-length recombinant human IKK β was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The IKK β gene accession number is [NM_001556](#).

Gene Aliases

IKK2, IKBKB, IKKB, NFKB1B, FLJ40509, IKK-beta, MGC131801

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

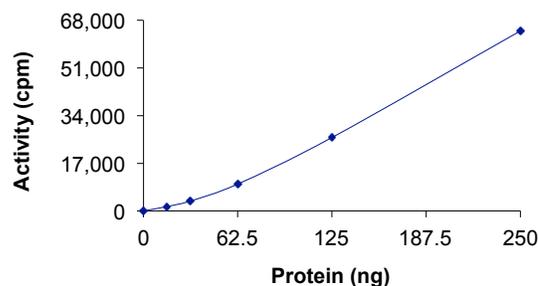
Scientific Background

IKK β is a serine/threonine protein kinase that phosphorylates the I-kappa-B protein which is an inhibitor of the transcription factor NF-kappa-B complex. Phosphorylation of I-kappa-B protein triggers the degradation of the inhibitor via the ubiquitination pathway, thereby activating NF-kappa-B complex. The activity of IKK β is stimulated by TNF and IL1 and IKK β forms a heterodimer that interacts with NIK (1). Overexpression of catalytically inactive IKK β blocks cytokine-induced NF-kappa-B activation. Aspirin and sodium salicylate can specifically inhibit IKK β activity in vitro and in vivo by binding to IKK β to reduce ATP binding (2).

References

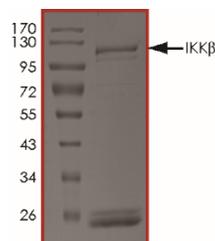
1. Woronicz, J D. et al: IkappaB kinase-beta: NF-kappa-B activation and complex formation with IkappaB kinase-alpha and NIK. *Science* 278: 866-869, 1997.
2. Yin, M.-J. et al: The anti-inflammatory agents aspirin and salicylate inhibit the activity of I-kappa-B kinase-beta. *Nature* 396: 77-80, 1998.

Specific Activity



The specific activity of IKK β was determined to be **18 nmol /min/mg** as per activity assay protocol.

Purity



The purity of IKK β was determined to be **>70%** by densitometry, approx. MW **~115kDa**.

IKK β , Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog #	103-18G
Specific Activity	18 nmol/min/mg
Lot #	U1536-6
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I03-18G)

Active IKK β (0.05 μ g/ μ l) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active IKK β for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/ μ l BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β -glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250 μ M [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 μ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 μ l [³³P]-ATP (1mCi/100 μ l), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200 μ l aliquots at -20°C.

Substrate (Catalog #: I33-58)

IKKtide synthetic peptide substrate (KKKKERLLDDRHDSG-LDSMKDEE) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active IKK β , Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μ l:
 - Component 1.** 10 μ l of diluted Active IKK β (Catalog #I03-18G)
 - Component 2.** 5 μ l of 1 mg/ml stock solution of substrate (Catalog #I33-58)
 - Component 3.** 5 μ l distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 μ l [³³P]-ATP Assay Cocktail bringing the final volume up to 25 μ l and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 μ l of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μ l [³³P]-ATP / pmoles of ATP (in 5 μ l of a 250 μ M ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/ μ g or nmol/min/mg)

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μ g or mg)]*[(Reaction Volume) / (Spot Volume)]

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