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1-866-9 KINASE (54-6273)

IKKβ, Active

Full length human recombinant protein expressed in Sf9 cells

Catalog # I03-10BG-10

Lot # F409-4

Product Description

Full length recombinant human IKKβ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The IKKβ gene accession number is NM_001556.

Gene Aliases

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

Concentration

0.1 µg/µl

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, Shipping 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.
Stability is 1yr at –70°C from date of shipment. Product shipped on dry ice.

Scientific Background

IKKβ 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 IKKβ is stimulated by TNF and IL1 and IKKβ forms a heterodimer that interacts with NIK (1). Overexpression of catalytically inactive IKKβ blocks cytokine-induced NF-kappa-B activation. Aspirin and sodium salicylate can specifically inhibit IKKβ activity in vitro and in vivo by binding to IKKβ 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.

Purity

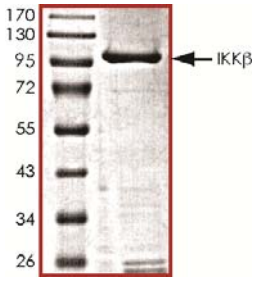
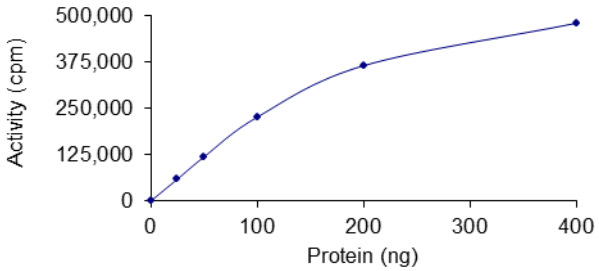


Figure 1. SDS-PAGE gel image

The purity of IKKβ was determined to be **>95%** by densitometry, approx. MW **~105kDa**.

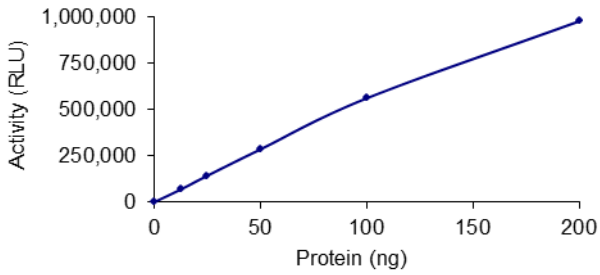
Specific Activity

Figure 2. Radiometric Assay Data



The specific activity of IKKβ was determined to be **58 nmol /min/mg** as per activity assay protocol.
(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of IKKβ was determined to be **95 nmol /min/mg** as per activity assay protocol.
(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I03-10BG)

Active IKKβ (0.1μg/μl) 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 IKKβ 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/μl BSA solution.

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

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33P}]-ATP (1mCi/100μl), 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 200μl aliquots at –20°C.

Substrate (Catalog #: I33-58)

IKKtide synthetic peptide substrate (KKKKERLLDDRHDSG-LDSMKDEE) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active IKKβ, 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 20μl:

- **Component 1.** 10μl of diluted Active IKKβ (Catalog #I03-10BG)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #I33-58)
- **Component 3.** 5μl distilled H_2O (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 H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl 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 20μl 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 H_2O) 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 [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5μl [^{33}P]-ATP / pmoles of ATP (in 5μl of a 250μM ATP stock solution, i.e., 1,250 pmoles)

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

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

ADP-Glo™ Activity Assay Protocol

Reaction Components

IKKβ Kinase Enzyme System (Promega, Catalog #:V4502)

IKKβ, Active, 10μg (0.1μg/μl)
IKKtide, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT solution (0.1M), 25μl

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)
ADP, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM $MgCl_2$ and 0.5 μg/μl BSA.

Assay Protocol

The IKKβ assay is performed using the IKKβ Kinase Enzyme System (Promega; Catalog #: V4502) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The IKKβ reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding t*he ADP-Glo™ Kinase Assay,* see the Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

Step 1. Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.

Step 2. Thaw the components of IKKβ Enzyme System, ADP and ATP on ice.

Step 3. Prepare 1ml of 2X Buffer by combining 400μl Reaction Buffer A, 1μl DTT and 599μl of dH_20.

Step 4. Prepare 1ml of 250μM ATP Assay Solution by adding 25μl ATP solution (10mM) to 500μl of 2X Buffer and 475μl of dH_20.

Step 5. Prepare diluted IKKβ in 1X Buffer (diluted from 2X buffer) 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 IKKB for optimal results).

Step 6. In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20μl:

Component 1.	10μl of diluted Active IKKβ
Component 2.	5μl of 1mg/ml stock solution of substrate
Component 3.	5μl of 2X Buffer

Step 7. Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 8. At the same time as the IKKβ kinase reaction, set up an ATP to ADP conversion curve at 50μM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* Technical Manual #TM313.

Step 9. Initiate the IKKβ reactions by the addition of 5μl of 250μM ATP Assay Solution thereby bringing the final volume up to 25μl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.

Step 10. Terminate the reaction and deplete the remaining ATP by adding 25μl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.

Step 11. Add 50μl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.

Step 12. Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).

Step 13. Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at http://www.promega.com/applications/cellularanalysis/cellsignaling.htm

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)