

## IKK $\alpha$ , Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # C51-10G-10**

Lot # N258-1

### Product Description

Full length recombinant human IKK $\alpha$  was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC092514](#).

### Gene Aliases

CHUK, IKK1, IKBKA, TCF16, NFKBIKA, IKK-alpha

### Concentration

0.1  $\mu\text{g}/\mu\text{l}$

### 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  $-70^{\circ}\text{C}$ . 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 6 months at  $-70^{\circ}\text{C}$  from date of shipment. Product shipped on dry ice.

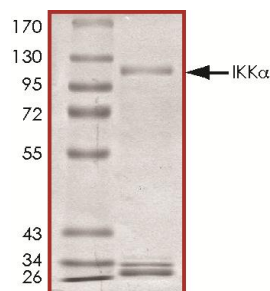
### Scientific Background

IKK $\alpha$  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 (1). IKK $\alpha$  is an essential regulator of NF-kappa-B-dependent gene expression through control of promoter-associated histone phosphorylation after cytokine exposure (2). IKK $\alpha$  is a critical component of the cytoplasmic transductional-transcriptional processor leading to induction of IFN $\alpha$  production. IKK $\alpha$  is also involved in the epidermis where it antagonizes mitogenic and angiogenic signals and represses tumor progression and metastases.

### References

1. Anest, V.et.al: A nucleosomal function for I-kappa-B kinase-alpha in NF-kappa-B-dependent gene expression. Nature 423: 659-663, 2003.
2. Hoshino, K. et.al: I-kappa-B kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. Nature 440: 949-953, 2006.

### Purity

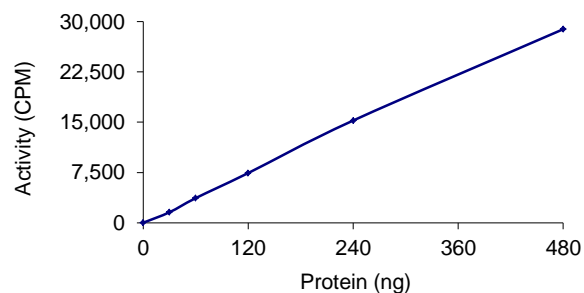


**Figure 1. SDS-PAGE gel image**

The purity of IKK $\alpha$  was determined to be **>75%** by densitometry, approx. MW **~114kDa**.

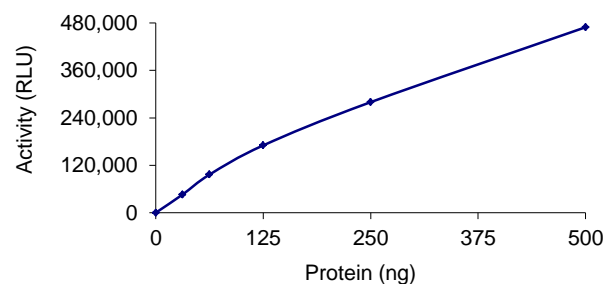
### Specific Activity

**Figure 2. Radiometric Assay Data**



The specific activity of IKK $\alpha$  was determined to be **2.5 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 IKK $\alpha$  was determined to be **15 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 #: C51-10G)

Active IKK $\alpha$  (0.1 $\mu$ g/ $\mu$ 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 $\alpha$  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 final 50ng/ $\mu$ l BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250 $\mu$ M [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150 $\mu$ l of 10mM ATP Stock Solution (Catalog #: A50-09), 100 $\mu$ l [<sup>33</sup>P]-ATP (1mCi/100 $\mu$ 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 $\mu$ l aliquots at -20°C.

### Substrate (Catalog #: I33-58)

IKKtide synthetic peptide substrate (KKKKERLLDDRHDSG-LDSMKDEE) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active IKK $\alpha$ , 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 $\mu$ l:
  - Component 1.** 10 $\mu$ l of diluted Active IKK $\alpha$  (Catalog #C51-10G)
  - Component 2.** 5 $\mu$ l of 1mg/ml stock solution of substrate (Catalog #I33-58)
  - Component 3.** 5 $\mu$ l distilled H<sub>2</sub>O (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<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 $\mu$ l [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25 $\mu$ 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 $\mu$ 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 H<sub>2</sub>O) 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 (cpm) 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 [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

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

### Kinase Specific Activity (SA) (pmol/min/ $\mu$ g or nmol/min/mg)

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

# ADP-Glo™ Activity Assay Protocol

## Reaction Components

### IKK $\alpha$ Kinase Enzyme System (Promega, Catalog #:V4068)

IKK $\alpha$ , Active, 10 $\mu$ g (0.1 $\mu$ g/ $\mu$ l)  
IKKtide, 1ml (1mg/ml)  
Reaction Buffer A (5X), 1.5ml  
DTT (0.1M), 25 $\mu$ l

### 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<sub>2</sub> and 0.5 mg/ml BSA.

## Assay Protocol

The IKK $\alpha$  assay is performed using the IKK $\alpha$  Kinase Enzyme System (Promega; Catalog #: V4068) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The IKK $\alpha$  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 the ADP-Glo™ Kinase Assay, see the technical Manual #TM313, available at [www.promega.com/tbs/tm313/tm313.html](http://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 IKK $\alpha$  Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400 $\mu$ l Reaction Buffer A, 1 $\mu$ l DTT and 599 $\mu$ l of dH<sub>2</sub>O.
- Step 4.** Prepare 1ml of 250 $\mu$ M ATP Assay Solution by adding 25 $\mu$ l ATP solution (10mM) to 500 $\mu$ l of 2X Buffer and 475 $\mu$ l of dH<sub>2</sub>O.
- Step 5.** Prepare diluted IKK $\alpha$  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 IKK $\alpha$  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 20 $\mu$ l:

<b>Component 1.</b>	10 $\mu$ l of diluted Active IKK $\alpha$
<b>Component 2.</b>	5 $\mu$ l of 1mg/ml stock solution of substrate
<b>Component 3.</b>	5 $\mu$ l 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<sub>2</sub>O.
- Step 8.** At the same time as the IKK $\alpha$  kinase reaction, set up an ATP to ADP conversion curve at 50 $\mu$ M ATP/ADP range as described in the ADP-Glo™ Kinase Assay technical Manual #TM313.
- Step 9.** Initiate the IKK $\alpha$  reactions by the addition of 5 $\mu$ l of 250  $\mu$ M ATP Assay Solution thereby bringing the final volume up to 25 $\mu$ l. 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 25 $\mu$ l 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 50 $\mu$ l 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 RLU, see Kinase Enzyme Systems Protocol at: <http://www.promega.com/KESProtocol>

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

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