

## Certificate of Analysis

### I $\kappa$ B $\alpha$ , activated

(Recombinant protein expressed in Sf21 insect cells)

Item # 23-028, 23-028-K, 23-028M

Parent Lot # D11EP043N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** N-terminal c-Myc, 6His-tagged, recombinant human I $\kappa$ B $\alpha$  full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Phosphorylated *in vitro* using IKK $\alpha$ , active (Item # 14-461)

Purity 70% by SDS-PAGE and Coomassie blue staining. MW = 41kDa.

**Formulation:** 0.221mg/ml of enzyme in 25mM MOPS pH7.5, 5mM MgCl<sub>2</sub>, 0.01% Tween 20, 1mM ATP. Frozen solution.

**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

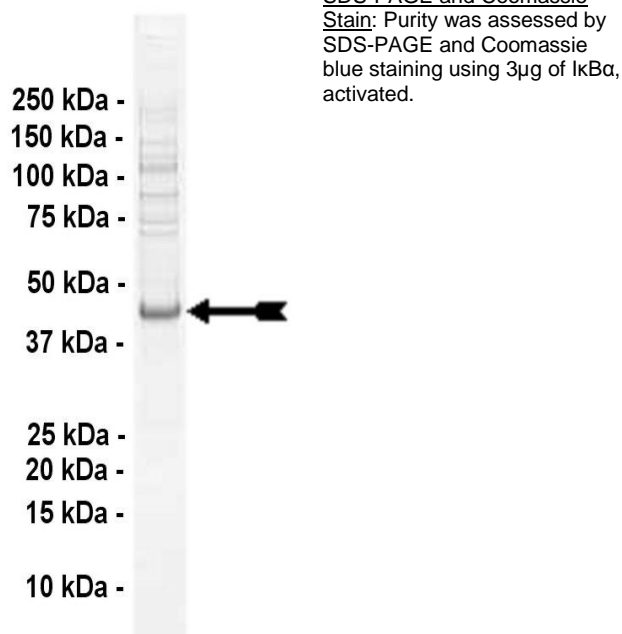
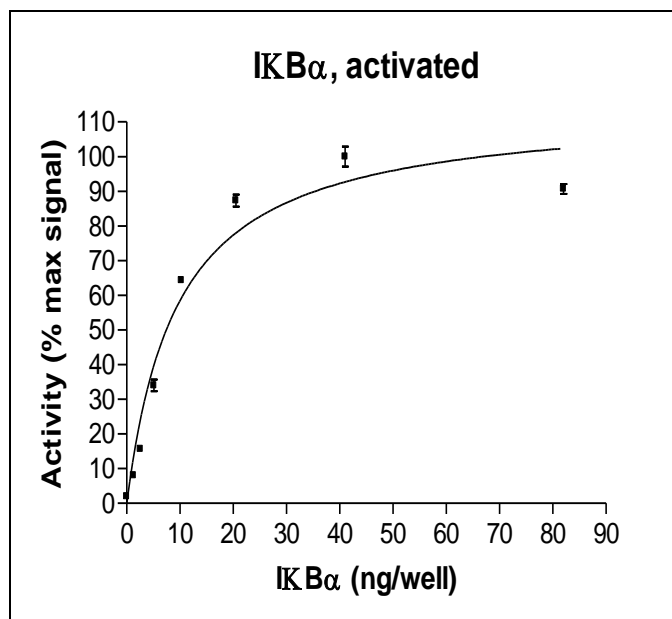
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Assay:** This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

**Protein Identity:** Confirmed identity as I $\kappa$ B $\alpha$  by mass spectrometry.



## Certificate of Analysis

### Assay Protocol

#### Reagents:

- |  |                           |
|--|---------------------------|
| 1. UBE1, active (Item # 23-021)                          | 4. 1x Reaction Buffer     |
| 2. UbcH3, active (Item # 23-022)                         | 5. Biotinylated-Ubiquitin |
| 3. SCF <sup>βTrCP1</sup> complex, active (Item # 23-026) | 6. Stop Solution          |

#### Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>).

IκBα, activated is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 10μM ATP, 10nM UBE1, 500nM UbcH3, 5μg/ml SCF<sup>βTrCP1</sup> complex, and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH 7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of IκBα is measured by detection of bound ubiquitin via electrochemiluminescence.

## IκBα Information

<b><u>Protein</u></b>	human IκBα
<b><u>Accession number</u></b>	GenBank M69043
<b><u>Alternative Names</u></b>	NF-kappa-B inhibitor alpha, I-kappa-B-alpha, Major histocompatibility complex enhancer-binding protein MAD3

<b><u>Key Facts</u></b>	IκBα is a cellular protein that inhibits the transcription factor NF-κB. NF-κB plays a central role in cellular stress and inflammatory responses by controlling cytokine-inducible gene expression and lymphocyte stimulation by antigens. In non-stimulated cells NF-κB is sequestered in the cytoplasm via the inhibitory protein IκBα, which obscures the nuclear localization signal of NF-κB and also blocks its ability to bind DNA. In response to various stimuli, including viral infection, ultraviolet radiation and inflammatory cytokines, IκBα is phosphorylated by the protein kinase complex IKK, which triggers its rapid degradation by ubiquitin mediated proteolysis. The liberated NF-κB then translocates to the nucleus and activates the expression of target genes. Mutations in the gene for IκBα result in the functional impairment of NF-κB which causes a decreased production of proinflammatory cytokines and certain interferons, rendering patients susceptible to infection.
-------------------------	--

<b><u>Related Products</u></b>	Item # 23-021 UBE1, active, Item # 23-022 UbcH3, active, Item # 23-025 UbcH4, active, Item # 23-026 SCF <sup>βTRCP1</sup> complex, active
--------------------------------	---

## **Selected References**

Ferreiro D.U. and Komives E. A. Molecular Mechanisms of System Control of NF-κB Signaling by IκBα. *Biochemistry*, 49: 1560-1567, 2010

Scherer D. C. *et al.*, Signal-Induced Degradation of IκBα Requires Site-Specific Ubiquitination. *PNAS*. 92: 11259-11263, 1995

Jungnickel B. *et al.*, Clonal Deleterious Mutations in the IκBα Gene in the Malignant Cells in Hodgkin's Lymphoma. *J Exp Med.*, 191: 395-402, 2000

Strack P. *et al.*, SCF<sup>β-TRCP</sup> and Phosphorylation Dependent Ubiquitination of IκBα Catalysed by Ubc3 and Ubc4. *Oncogene*, 19: 3529-3536, 2000

Winston J. T. *et al.*, The SCF<sup>β-TRCP</sup>-Ubiquitin Ligase Complex Associates Specifically with Phosphorylated Destruction Motifs in IκBα and β-catenin and Stimulates IκBα Ubiquitination *in vitro*. *Genes Dev*, 13: 270-283, 1999

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services