APA563Mu61 100µg Active Interleukin 1 Beta (IL1b) Organism Species: Mus musculus (Mouse) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Val118~Ser269 Tags: N-terminal His-tag Purity: >98% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300. Applications: Cell culture; Activity Assays; In vivo assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.4 Predicted Molecular Mass: 19.0kDa Accurate Molecular Mass: 19kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

VPI RQLHYRLRDE QQKSLVLSDP YELKALHLNG QNINQQVIFS MSFVQGEPSN DKIPVALGLK GKNLYLSCVM KDGTPTLQLE SVDPKQYPKK KMEKRFVFNK IEVKSKVEFE SAEFPNWYIS TSQAEHKPVF LGNNSGQDII DFTMESVSS

[ACTIVITY]

IL-1β (Interleukin-1 beta) is a proinflammatory and immunoregulatory cytokine involved in a variety of cellular activities. It has been elucidated that IL-1β stimulation of cells activates MMP-9 (matrix metalloproteinases-9) secretion by the activation of the dual signalling pathways. Thus, a stimulation assay of IL-1β was conducted using 3T3 cell line, and the MMP-9 activity was detected through gel zymography. Briefly, 1×10^6 3T3 cells were cultured overnight in 5%DMEM, washed with serum-free medium and then stimulated with different concentrations of IL-1β for 20h, and cell lysates were collected to measure MMP-9 activity. Cell lysates samples were denatured by SDS loading buffer, electrophoresed through sodium dodecyl sulphate–polyacrylamide gel (SDS–PAGE; 10% gels) containing gelatin (1mg/mL) with nonreducing conditions. After renaturation, incubation and coomassiebrilliant blue (CBB)-stained, MMPs hydrolyzed gelatin nearby, indicated by the white binds on the gel.

Result: Increased MMP-9 activity in 3T3 cells due to the stimulation of IL-1 β was shown in Figure 1.

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Figure 1. Activation of MMP-9 by IL-1β.

[IDENTIFICATION]

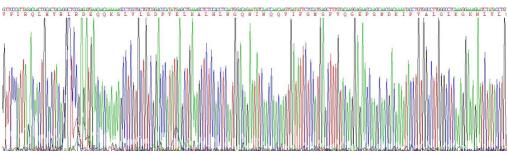


Figure 2. Gene Sequencing (extract)

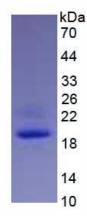


Figure 3. SDS-PAGE

Sample: Active recombinant IL1b, Mouse

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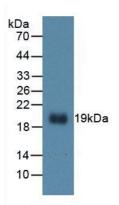


Figure 4. Western Blot

Sample: Recombinant IL1b, Mouse;

Antibody: Rabbit Anti-Mouse IL1b Ab (PAA563Mu06)