APA073Mu61 100µg Active Interleukin 2 (IL2) Organism Species: Mus musculus (Mouse) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ala21~Gln169

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.

Predicted isoelectric point: 4.9

Predicted Molecular Mass: 18.9kDa

Accurate Molecular Mass: 25kDa as determined by SDS-PAGE reducing conditions.

Applications: Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
- 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
- 5. Polymerization of the target protein: Dimerization, multimerization etc.

[<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.

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.

[<u>SEQUENCE</u>]

APTSSSTSSS TAEAQQQQQQ QQQQQQHLEQ LLMDLQELLS RMENYRNLKL PRMLTFKFYL PKQATELKDL QCLEDELGPL RHVLDLTQSK SFQLEDAENF ISNIRVTVVK LKGSDNTFEC QFDDESATVV DFLRRWIAFC QSIISTSPQ

[ACTIVITY]

Interleukin-2 (IL-2), the main lymphokine produced by activated CD4⁺ T-helper lymphocytes, is a potent T-cell growth regulator. In addition to this activity, IL-2 was found to decrease the proliferation of 3T3 fibroblasts in time- and dose-dependent manner. Thus 3T3 cells were seeded overnight at a density of 5,000 cells/well, and treated with or without various concentrations of IL2 for 48h, then 3T3 cells were observed by inverted microscope and cell viability was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ L of CCK-8 solution was added to each well of the plate, then measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37°C.

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The inhibitory effect of IL-2 on cell proliferation of 3T3 cells observed by inverted microscope was shown in Figure 1.

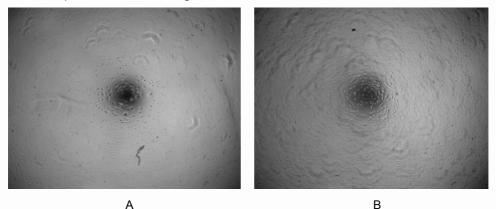
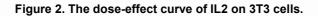


Figure 1. The inhibitory effect of IL-2 on cell proliferation of 3T3 cells. (A) 3T3 cells cultured in DMEM, stimulated with IL2 (10ng/mL) for 72h; (B) Unstimulated 3T3 cells cultured in DMEM for 72h.

The dose-effect curve of IL2 was shown in Figure 2. It was obvious that IL2 significantly decreased cell growth of 3T3 cells. The ED50 for this effect is typically 9.876 to 13.35ng/mL.

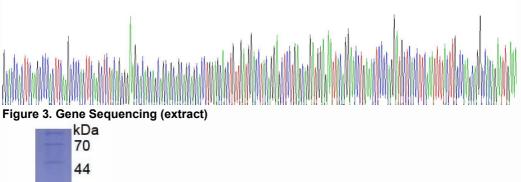




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[IDENTIFICATION]

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10 Figure 4. SDS-PAGE

Sample: Active recombinant IL2, Mouse

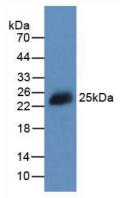


Figure 5. Western Blot

Sample: Recombinant IL2, Mouse;

Antibody: Rabbit Anti-Mouse IL2 Ab (PAA073Mu06)