#### APA073Mu61 5mg Active Interleukin 2 (IL2) 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: Ala21~Gln169 Tags: N-terminal His-tag

**Purity: >95%** 

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% saccharose.

Original Concentration: 400µg/mL

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: 4.9

Predicted Molecular Mass: 18.9kDa

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

#### 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 10mM PBS (pH7.4) 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]

IL-2(Interleukin-2) is a cytokine produced by T-cells in response to antigenic or mitogenic stimulation. IL-2 is a type of signaling molecule in the immune system, that is required for both T-cell and B-cell proliferation and other activities crucial to regulation of the immune response. Therefore, in order to detect the bioactivity of recombinant mouse IL-2, spleen single suspensions were prepared, activated with conA (final concentration 3 ug/ml). Cells were collected after 72h and washed with hanks. Then mouse splenic lymphocytes were were seeded into triplicate wells of 96-well plates at a density of 10,000 cells/well with or without the addition of various concentrations of recombinant mouse IL-2. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8(CCK-8). 10  $\mu$ l of CCK-8 solution was added to each well of the plate, the absorbance at 450 nm was measured using a microplate reader after

incubating the plate for 1-4 hours at 37 °C . Proliferation of Splenic lymphocytes cells after incubation with IL-2 for 72h observed by inverted microscope was shown in Figure 1







(A) Splenic lymphocytes cells cultured in 1640, stimulated with 1ng/ml IL-2 for 72h;

(B)Unstimulated Splenic lymphocytes cells cultured in 1640 for72h.

The dose-effect curve of recombinant mouse IL-2 was shown in Figure 2. It was obvious that recombinant mouse IL-2 significantly promoted cell proliferation of Splenic lymphocytes cells .The ED50 for this effect is typically 0.7-1.7ng/ml.

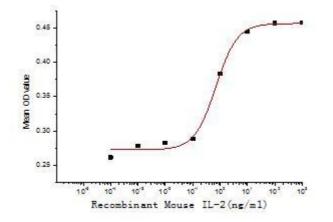
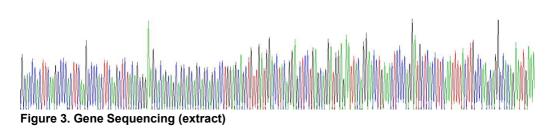
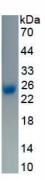


Figure 2. The dose-effect curve of IL-2 on Splenic lymphocytes cells

# [IDENTIFICATION]



COLOCULTICMECCICITICMECCICITAL COCOLICITICAL COLORGE ACCULCULAR CALCULAR C





Sample: Active recombinant IL2, Mouse

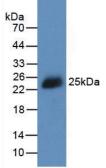


Figure 5. Western Blot

Sample: Recombinant IL2, Mouse;

Antibody: Rabbit Anti-Mouse IL2 Ab (PAA073Mu06)

## [<u>IMPORTANT NOTE</u>]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.