APA077Hu61 50µg Active Interleukin 4 (IL4) Organism Species: Homo sapiens (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: His25~Ser153 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 5% Trehalose. Original Concentration: 50µg/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: 9.3 Predicted Molecular Mass: 16.6kDa Accurate Molecular Mass: 20kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in ddH₂O to a concentration of 0.1-0.5 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.

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[<u>SEQUENCE</u>]

HKCDIT LQEIIKTLNS LTEQKTLCTE LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKYSK CSS

[ACTIVITY]

The interleukin 4 (IL4,) is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL4, Th2 cells subsequently produce additional IL4 in a positive feedback loop. IL4 has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL4 induces B-cell class switching to IgE, and up-regulates MHC class II production. IL4 decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL12. The activity of IL4 is usually measured by a cell proliferation assay using TF-1 cells. TF-1 cells were seeded into triplicate wells of 96-well plates at a density of 20000 cells/well with 10% serum standard 1640 which contains various concentrations of recombinant human IL4. After incubated for 3 days, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 µl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 2-4 hours at 37 $^{\circ}$ C. Proliferation of TF-1 cells after incubation with IL4 for 3 days observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with rhIL4 for 3 days. The result was shown in Figure 2. It was obvious that rhIL4 significantly increased cell viability of TF-1 cells. The ED50 is 0.03 ng/ml.

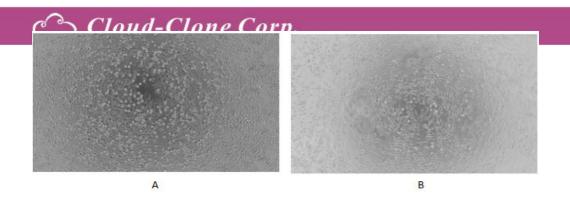


Figure 1. Cell proliferation of TF-1 cells after stimulated with IL4.

(A) TF-1 cells cultured in 1640, stimulated with 0.1 ng/ml rhlL4 for 3 days;(B) Unstimulated TF-1 cells cultured in 1640 for 3 days.

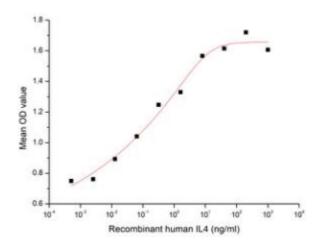
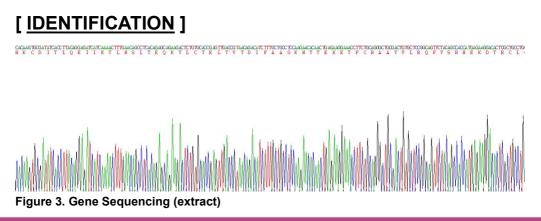


Figure 2. Cell proliferation of TF-1 cells after stimulated with rhIL4.



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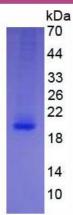


Figure 4. SDS-PAGE

Sample: Active recombinant IL4, Human

[<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.