

APA077Ca61 100µg
Active Interleukin 4 (IL4)

Organism Species: *Canis familiaris*; Canine (Dog)
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: Gly24~His132

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: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 10.3

Predicted Molecular Mass: 14.4kDa

Accurate Molecular Mass: 18-33kDa 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.

[USAGE]

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.

[SEQUENCE]

GHNFNITIKEIKMLNILTARNDSCELTVKDVFTAPKNTSDKEIFCRAATVLRQIYTHNCSNRYLR
GLYRNLSSMANKTCSMNEIKKSTLKDFLERLKVIMQKKYYRH

[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 μ l 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 4.2 ng/ml.

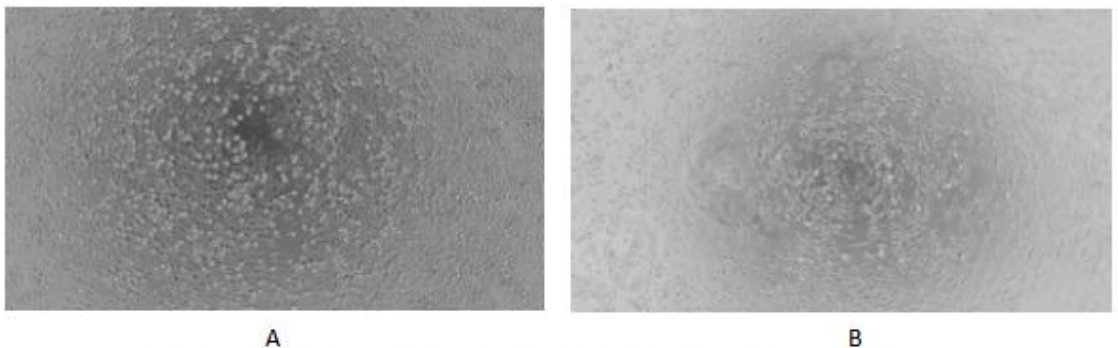


Figure 1. Cell proliferation of TF-1 cells after stimulated with IL4.

- (A) TF-1 cells cultured in 1640, stimulated with 10 ng/ml rdIL4 for 3 days;
- (B) Unstimulated TF-1 cells cultured in 1640 for 3 days.

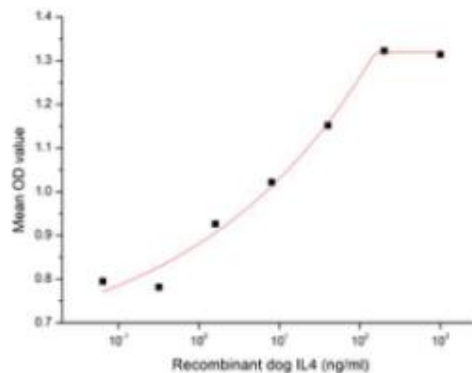


Figure 2. Cell proliferation of TF-1 cells after stimulated with rdIL4.

[IDENTIFICATION]

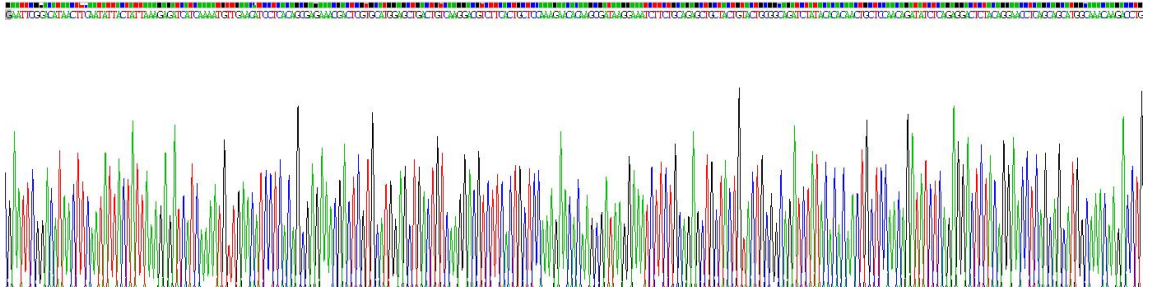


Figure 3. Gene Sequencing (extract)

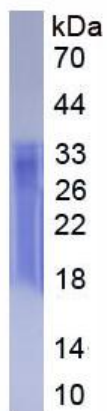


Figure 4. SDS-PAGE

Sample: Active recombinant IL4, Dog

[IMPORTANT NOTE]

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.