APA056Ga61 200µg Active Interleukin 10 (IL10) Organism Species: *Chicken (Gallus) 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: Leu22~Lys175 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: 6.7 Predicted Molecular Mass: 19.9kDa Accurate Molecular Mass: 20kDa as determined by SDS-PAGE reducing conditions.

[<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.

[SEQUENCE]

LEPTCLHFS ELLPARLREL RVKFEEIKDY FQSRDDELNI QLLSSELLDE FKGTFGCQSV SEMLRFYTDE VLPRAMQTST SHQQSMGDLG NMLLGLKATM RRCHRFFTCE KRSKAIKQIK ETFEKMDENG IYKAMGEFDI FINYIEEYLL MRRRK

[ACTIVITY]

Interleukin 10, also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 family of alpha - helical cytokines that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155. Mature human IL-10 shares 72%-86% amino acid sequence identity with bovine, canine, equine, feline, mouse, ovine, porcine, and rat IL-10. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells. IL-10 is a critical molecule in the control of viral infections and allergic and autoimmune inflammation. It promotes phagocytic uptake and Th2 responses but suppresses antigen presentation and Th1 proinflammatory responses. The activity of IL10 is usually measured by a cell proliferation assay using MC/9 mouse mast cells. MC/9 cells were seeded into triplicate wells of 96-well plates at a density of 8,000 cells/well with 2% serum standard DMEM which contains various concentrations of recombinant chicken 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 °C. Proliferation of MC/9 cells after incubation with IL10 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 recombinant chicken IL10 for 3 days.

The result was shown in Figure 2. It was obvious that IL10 significantly increased cell viability of MC/9 cells. The ED50 is 0.92 -9.2 ng/ml.



Figure 1. Cell proliferation of MC/9 cells after stimulated with IL10.

(A) MC/9 cells cultured in DMEM, stimulated with 10 ng/ml IL10 for 3 days;

(B) Unstimulated MC/9 cells cultured in DMEM for 3 days.



Figure 2. Cell proliferation of MC/9 cells after stimulated with IL10.



Figure 3. Gene Sequencing (extract)



Figure 4. SDS-PAGE

Sample: Active recombinant IL10, Gallus

[<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.