Coud-Clone Corp.

APA079Ca01 100µg Active Interleukin 6 (IL6) Organism Species: *Canis familiaris; Canine* (Dog) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Ala18~Met207 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.5 Predicted Molecular Mass: 24.9kDa Accurate Molecular Mass: 25kDa as determined by SDS-PAGE reducing conditions. [USAGE]

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.

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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]

ATA FPTPGPLAGD SKDDATSNSL PLTSANKVEE LIKYILGKIS ALRKEMCDKF NKCEDSKEAL AENNLHLPKL EGKDGCFQSG FNQETCLTRI TTGLVEFQLH LNILQNNYEG DKENVKSVHM STKILVQMLK SKVKNQDEVT TPDPTTDASL QAILQSQDEC VKHTTIHLIL RSLEDFLQFS LRAVRIM

[ACTIVITY]

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. Interleukin 6 is secreted by T cells and macrophages to stimulate immune response and also plays a role in fighting infection. It supports the growth of B cells and is antagonistic to regulatory T cells. To test the effect of IL6 on cell proliferation, Raji cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well with 1% serum standard 1640 which contains various concentrations of recombinant dog IL6. After incubated for 5 days, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10µL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of Raji cells after incubation with IL6 for 5 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 IL6 for 5 days. The result was shown in Figure 2. It was obvious that IL6 significantly increased cell viability of Raji cells.

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Figure 1. Cell proliferation of Raji cells after stimulated with IL6.

(A) Raji cells cultured in 1640, stimulated with 1ng/mL IL6 for 5 days;

(B) Unstimulated Raji cells cultured in 1640 for 5 days.

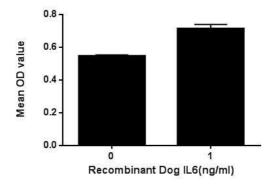


Figure 2. Cell proliferation of Raji cells after stimulated with IL6.



[IDENTIFICATION]

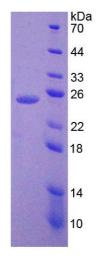


Figure 3. SDS-PAGE

Sample: Active recombinant IL6, Canine

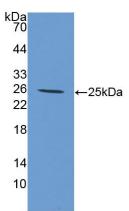


Figure 4. Western Blot Sample: Recombinant IL6, Canine; Antibody: Rabbit Anti-Canine IL6 Ab (PAA079Ca01)

[IMPORTANT NOTE]

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