

APA079Hu61 100µg

Active Interleukin 6 (IL6)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Val30~Met212

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.

Predicted isoelectric point: 6.2

Predicted Molecular Mass: 22.4kDa

Accurate Molecular Mass: 22&24kDa as determined by SDS-PAGE reducing conditions.

Applications: Cell culture; Activity Assays; In vivo assays.

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

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 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.

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]

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V PPGEDSKDVA APHRQPLTSS  
ERIDKQIRYI LDGISALRKE TCNKSNCES SKEALAENNL NLPKMAEKDG  
CFQSGFNEET CLVKIITGLL EFEVYLEYLQ NRFESSEEQA RAVQMSTKVL  
IQFLQKKAKN LDAITTPDPT TNASLLTKLQ AQNQWLQDMT THLILRSFKE  
FLQSSLRALR QM
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[ACTIVITY]

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. Current data suggest that direct application of IL-6 on breast cancer cells inhibits proliferation in ER-positive (estrogen- receptor-positive) cells through the Jak/Stat3 pathway. To test the inhibitory effect of IL-6 on proliferation of ER-positive MCF-7 cell line, cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of IL-6. After incubated for 96h, 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 measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37 $^{\circ}$ C.

The inhibitory effect of IL-6 on cell proliferation of MCF-7 cells observed by inverted microscope and detected by CCK-8 was shown in Figure 1 and Figure 2 respectively (Dose-dependent effect was not detected in this case).

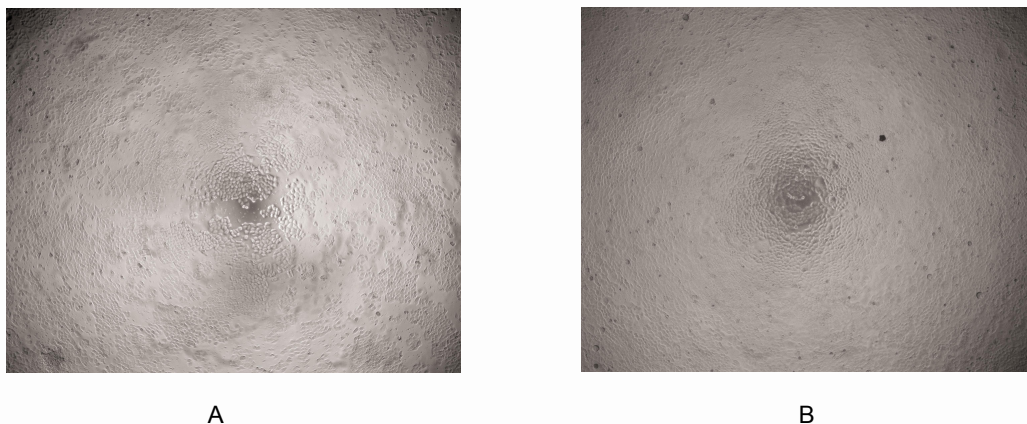


Figure 1. The inhibitory effect of IL-6 on cell proliferation of MCF-7 cells .

- (A) MCF-7 cells cultured in serum-free DMEM, stimulated with 100ng/mL IL-6 for 96h;**
(B) Unstimulated MCF-7 cells cultured in serum-free DMEM for 96h.

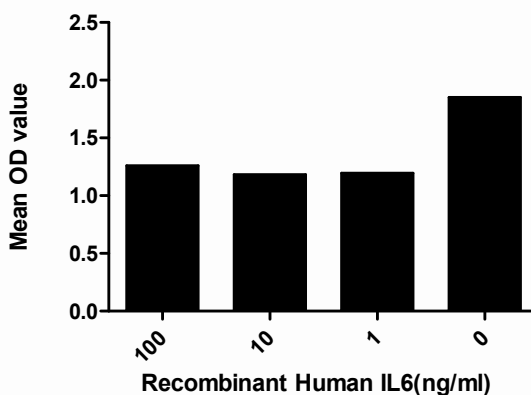


Figure 2. The inhibitory effect of IL-6 on cell proliferation of MCF-7 cells detected by CCK8.

[IDENTIFICATION]

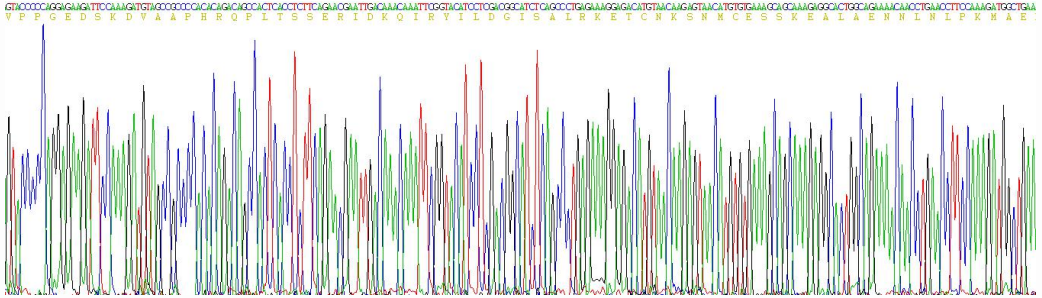


Figure 3. Gene Sequencing (extract)

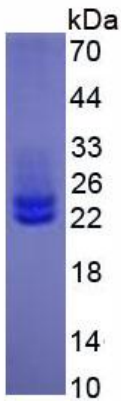


Figure 4. SDS-PAGE

Sample: Active recombinant IL6, Human

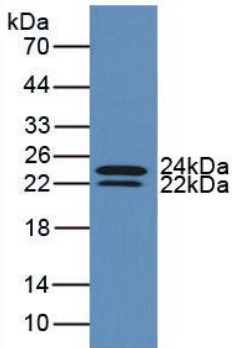


Figure 5. Western Blot

Sample: Recombinant IL6, Human;

Antibody: Rabbit Anti-Human IL6 Ab (PAA079Hu06)