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APA079Po61 100µg Active Interleukin 6 (IL6) Organism Species: *Sus scrofa; Porcine (Pig) 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: Gly30~Met212

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: 5.8

Predicted Molecular Mass: 22.5kDa

Accurate Molecular Mass: 25&30kDa 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.

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[<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]

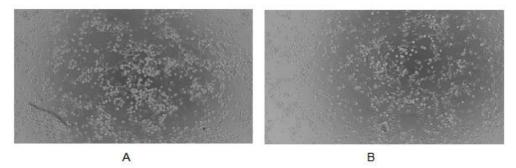
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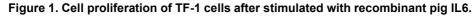
[ACTIVITY]

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine. 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, TF-1 cells were seeded into triplicate wells of 96-well plates at a density of 8,000 cells/well with 2% serum standard 1640 which contains various concentrations of recombinant pig IL6. After incubated for 48h, 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 1-4 hours at 37 $^{\circ}$ C. Proliferation of TF-1 cells after incubation with IL6 for 48h observed by

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inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant pig IL6 for 48h. The result was shown in Figure 2. It was obvious that IL6 significantly increased cell viability of TF-1 cells.





- (A) TF-1 cells cultured in 1640, stimulated with 1 ug/ml IL6 for 48h;
- (B) Unstimulated TF-1 cells cultured in 1640 for 48h.

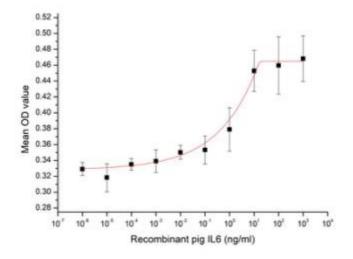


Figure 2. Cell proliferation of TF-1 cells after stimulated with IL6.

[IDENTIFICATION]

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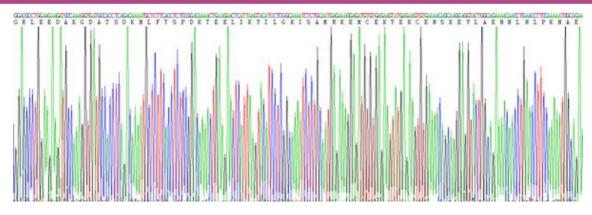


Figure 3. Gene Sequencing (extract)

_	kDa 70
	44
	33
-	26
	22
	18
	14
	10

Figure 4. SDS-PAGE

Sample: Active recombinant IL6, Pig

[<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.