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APA076Mu61 100µg Active Interleukin 3 (IL3) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Asp33~Cys166 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. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 7.9 Predicted Molecular Mass: 16.7kDa Accurate Molecular Mass: 25kDa 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.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.6) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

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[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]

DTHRLTRT LNCSSIVKEI IGKLPEPELK TDDEGPSLRN KSFRRVNLSK FVESQGEVDP EDRYVIKSNL QKLNCCLPTS ANDSALPGVF IRDLDDFRKK LRFYMVHLND LETVLTSRPP QPASGSVSPN RGTVEC

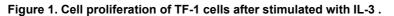
[ACTIVITY]

Interleukin 3 is an interleukin, a type of biological signal that can improve the body's natural response to disease as part of the immune system. It acts by binding to the interleukin 3 receptor IL-3 synergizes with other cytokines to stimulate the growth of immature progenitor cells of all lineages, and is therefore a multi-lineage colony-stimulating factor (CSF). It prevents cell death and promotes the survival of macrophages, mast cells, and megakaryocytes. To test the effect of IL-3 on cell proliferation, TF-1 cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well with 2% serum standard 1640 which contains various concentrations of recombinant mouse IL-3. After incubated for 72h, 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 IL-3 for72h observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8(Cell Counting Kit-8) assay after

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incubation with recombinant IL-3 for 72h. The result was shown in Figure2. It was obvious that IL-3 significantly increased cell viability of TF-1 cells. The ED50 is 3.79 ng/ml.





- (A) TF-1 cells cultured in RPMI-1640, stimulated with 100ng/ml IL-3 for 72h;
- (B) Unstimulated TF-1 cells cultured in RPMI-1640 for 72h.

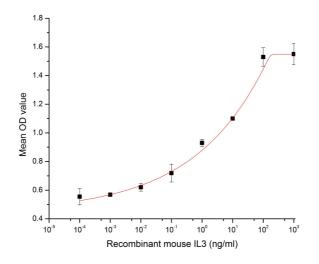
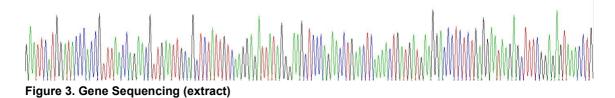


Figure 2. The dose-effect curve of IL-3 on TF-1 cells

[IDENTIFICATION]

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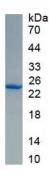


Figure 4. SDS-PAGE

Sample: Active recombinant IL3, Mouse

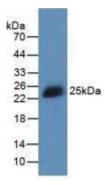


Figure 5. Western Blot

Sample: Recombinant IL3, Mouse;

Antibody: Rabbit Anti- Mouse IL3 Ab (PAA076Mu06)

[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.