

APA028Ca61 100µg
Active Erythropoietin (EPO)
Organism Species: *Canis familiaris*; Canine (Dog)
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: Ala41~Arg206

Tags: N-terminal His-tag

Purity: >90%

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: 7.6

Predicted Molecular Mass: 20.0kDa

Accurate Molecular Mass: 35kDa 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.

[USAGE]

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]

APPRLICDSRVLERYILEAREAENVTMGCAQGCSFSENITVPDTKVNIFYTWKRMDVGQQALEVWQGLALLSEAILRGQALLANASQPSET
QLHVDKAVSSLRSLTSLLRALGAQKEAMSLPEEASPAPLRTFTVDTLCKLFRIYSNFLRGKLTLYTGEACRRGDR

[ACTIVITY]

Erythropoietin (EPO) is a growth factor produced in the kidneys that stimulates the production of red blood cells. It works by promoting the division and differentiation of committed erythroid progenitors in the bone marrow. Epo additionally plays a tissue protective role in ischemia by blocking apoptosis and inducing angiogenesis.

To test the effect of EPO 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 dog EPO. After incubated for 48h, 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 4 hours at 37 °C. Proliferation of TF-1 cells after incubation with EPO for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 assay

after incubation with recombinant dog EPO for 48h. The result was shown in Figure 2. It was obvious that EPO significantly increased cell viability of TF-1 cells. The ED50 is 17 ng/ml.

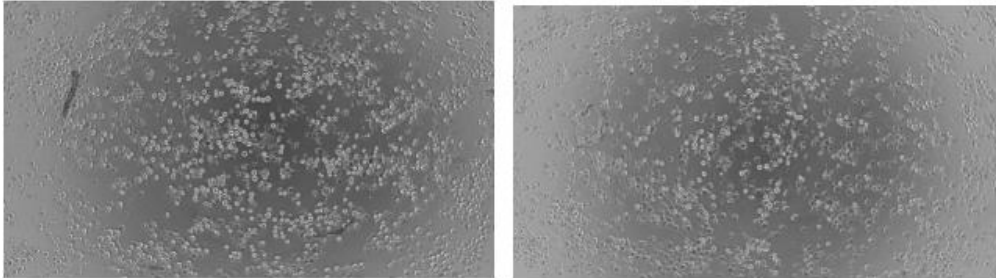


Figure 1. Cell proliferation of TF-1 cells after stimulated with recombinant dog EPO.

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

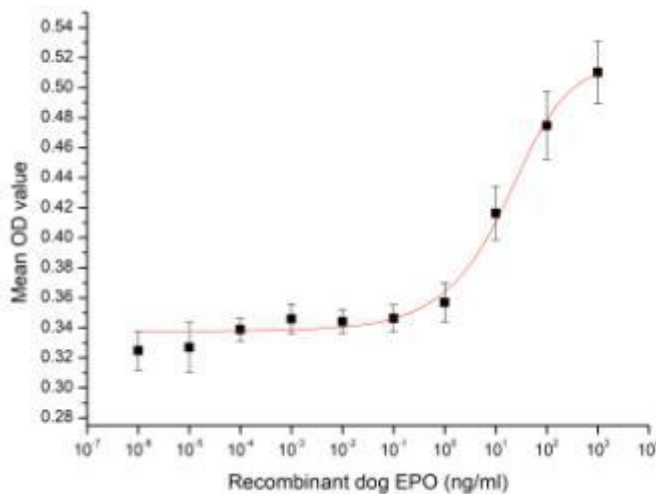


Figure 2. Cell proliferation of TF-1 cells after stimulated with recombinant dog EPO.

[IDENTIFICATION]

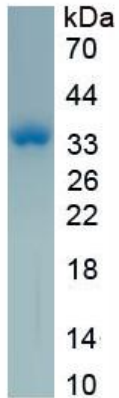


Figure 3. SDS-PAGE

Sample: Active recombinant EPO, Dog

[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.