APA028Hu61 100μg
Active Erythropoietin (EPO)
Organism Species: *Homo sapiens* (Human)
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: Ala28~Arg193
Tags: N-terminal His-tag
Purity: >95%
Buffer Formulation: 10mM PBS, pH7.6, 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: 8.8
Predicted Molecular Mass: 20.0kDa
Accurate Molecular Mass: 33-40kDa 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 (pH7.6) 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]

```
                                   APP RLICDSRVLE RYLLEAKEAE
NITTGCAEHC SLNENITVPD TKVNFYAWKR MEVGQQAVEV WQGLALLSEA
VLRGQALLVN SSQPWEPLQL HVDKAVSGLR SLTTLLRALG AQKEAISPPD
AASAAPLRTI TADTFRKLFR VYSNFLRGKL KLYTGEACRT GDR
```

[ACTIVITY]

Erythropoietin (EPO), also known as hematopoietin or hemopoietin, is a glycoprotein cytokine secreted by the kidney in response to cellular hypoxia. Erythropoietin is an essential hormone for red blood cell production. Its primary effect on red blood cell progenitors and precursors (which are found in the bone marrow in humans) by promoting their survival through protecting these cells from apoptosis, or cell death. EPO is the primary erythropoietic factor that cooperates with various other growth factors involved in the development of erythroid lineage from multipotent progenitors. Besides, Erythropoietin Receptor (EPOR) has been identified as an interactor of EPO, thus a binding ELISA assay was conducted to detect the interaction of recombinant human EPO and recombinant human EPOR. Briefly, EPO were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to EPOR-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with

anti-EPO pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of EPO and EPOR was shown in Figure 1, and this effect was in a dose dependent manner.

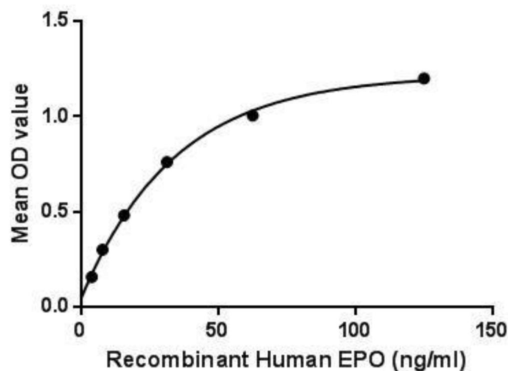

Figure 1. The binding activity of EPO with EPOR.

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 5,000 cells/well with 1% serum standard 1640 including various concentrations of recombinant human EPO. After incubated for 72h, 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℃. Proliferation of TF-1 cells after incubation with EPO for 72h 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 EPO for 72h. The result was shown in Figure 2. It was obvious that EPO significantly increased cell viability of TF-1 cells.

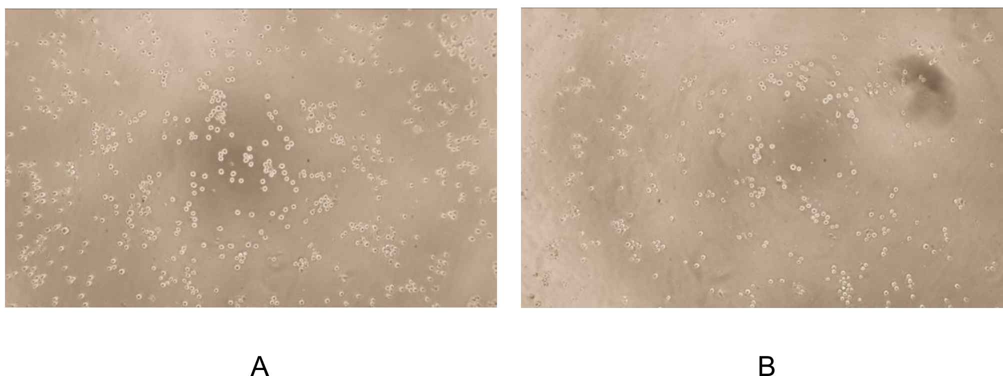

Figure 2. Cell proliferation of TF-1 cells after stimulated with EPO.

(A) TF-1 cells cultured in 1640, stimulated with 50ng/mL EPO for 72h;

(B) Unstimulated TF-1 cells cultured in1640 for 72h.

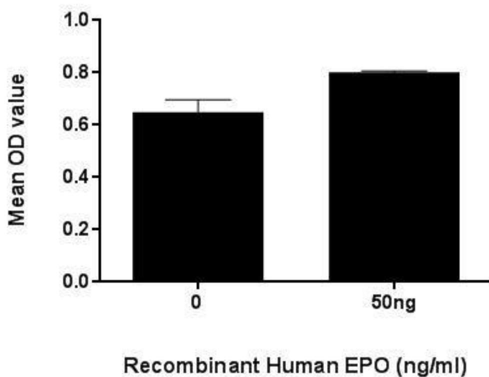

Figure 3. Cell proliferation of TF-1 cells after stimulated with EPO.

[IDENTIFICATION]

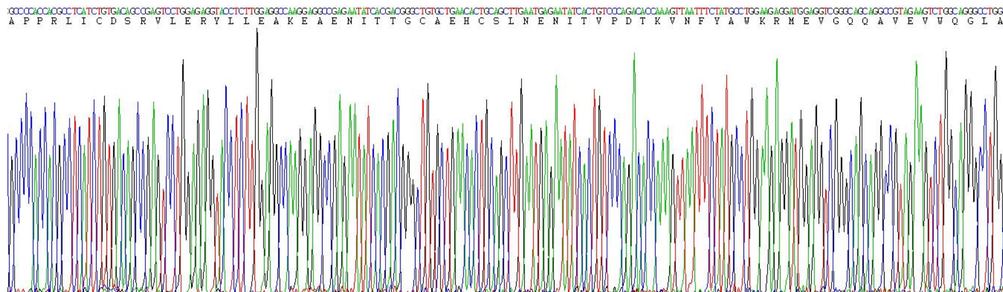

Figure 4. Gene Sequencing (extract)

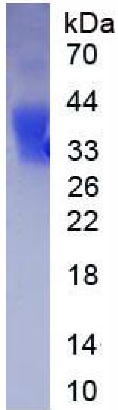

Figure 5. SDS-PAGE

Sample: Active recombinant EPO, Human

[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.