APA846Hu61 100μg
Active Prolactin (PRL)
Organism Species: Homo sapiens (Human)
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: Leu29~Cys227

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: 400μg/mL

Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.2

Predicted Molecular Mass: 23.4kDa

Accurate Molecular Mass: 24&28kDa 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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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]

LP ICPGGAARCQ VTLRDLFDRA
VVLSHYIHNL SSEMFSEFDK RYTHGRGFIT KAINSCHTSS LATPEDKEQA
QQMNQKDFLS LIVSILRSWN EPLYHLVTEV RGMQEAPEAI LSKAVEIEEQ
TKRLLEGMEL IVSQVHPETK ENEIYPVWSG LPSLQMADEE SRLSAYYNLL
HCLRRDSHKI DNYLKLLKCR IIHNNNC

[ACTIVITY]

PRL (prolactin), also known as luteotropin, is a hormone secreted from the pituitary gland and is best known for its role in enabling mammals to produce milk. PRL plays an essential role in metabolism, regulation of the immune system. According to reports, PRL also stimulates proliferation of certain cells, including MCF-7. Thus, proliferation assay of PRL was conducted using MCF-7 cells. Briefly, MCF-7 cells were seeded into triplicate wells of 96-well plates at a density of 2,000cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of PRL. 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 37ºC.

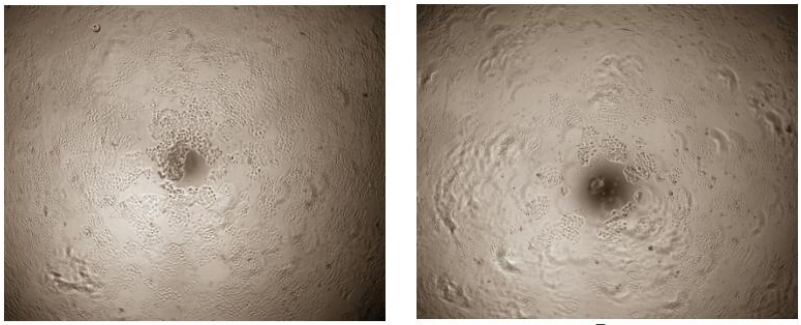

Figure 1. Cell proliferation of MCF-7 cells after stimulated with PRL.

(A) MCF-7 cells cultured in DMEM, stimulated with 1ng/mL PRL for 72h;

(B) Unstimulated MCF-7 cells cultured in DMEM for 72h.

The dose-effect curve of PRL was shown in Figure 2. It was obvious that PRL significantly promoted cell proliferation of MCF-7 cells. The ED50 for this effect is typically 3.709 to 8.973 ng/mL.

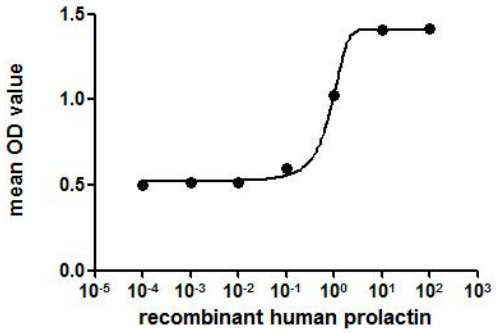

Figure 2. The dose-effect curve of PRL on MCF-7 cells.

PRL (prolactin), also known as luteotropin, is a hormone secreted from the pituitary gland and is best known for its role in enabling mammals to produce milk. PRL plays an essential role in metabolism, regulation of the immune system through activating its specific membrane-anchored receptor (PRLR). A functional ELISA assay was conducted to detect the interaction of recombinant human PRL and recombinant human PRLR. Briefly, PRL was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to

PRLR-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-PRL pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37℃, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 μL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human PRL and recombinant human PRLR was shown in Figure 1, and this effect was in a dose dependent manner.

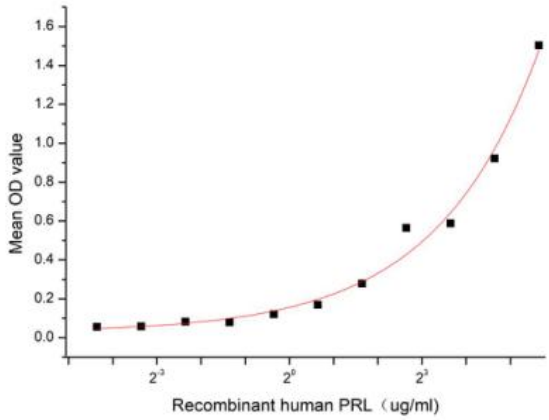

Figure 3. The binding activity of recombinant human PRL and recombinant human PRLR

[IDENTIFICATION]

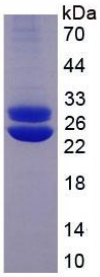

Figure 4. SDS-PAGE

Sample: Active recombinant PRL, Human

[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.