Coud-Clone Corp.

APA866Ra01 100µg Active Parathyroid Hormone (PTH) Organism Species: Rattus norvegicus (Rat) *Instruction manual* 

#### FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

### [PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Ala32~Gln115

Tags: Two N-terminal Tags, His-tag and MBP-tag

**Purity: >92%** 

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

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: 5.8

Predicted Molecular Mass: 59.4kDa

Accurate Molecular Mass: 59kDa as determined by SDS-PAGE reducing conditions.

### [ <u>USAGE</u> ]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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.

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**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 ]

#### AVSEIQLMH NLGKHLASVE RMQWLRKKLQ DVHNFVSLGV QMAAREGSYQ RPTKKEENVL VDGNSKSLGE GDKADVDVLV KAKSQ [ACTIVITY]

PTH (Parathyroid hormone) is a hormone secreted by the parathyroid glands that is important in bone remodeling. As reported, osteoblast-like cell lines, such as ROS 17/2.8, UMR106, SaOS, U2OS, MG63, that exhibit PTHR1, respond with increased proliferation to PTH. Rat PTH shares similarities with human PTH in amino acid sequence with the identity of 71.3%. Thus, a proliferation assay of rat recombinant PTH was conducted using U2OS cells. Briefly, U2OS cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of PTH. After incubated for 48h, 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°C. Proliferation of U2OS cells after incubation with PTH for 48h 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 PTH for 48h. The result was shown in Figure 2. It was obvious that PTH increased cell viability of U2OS cells.

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Figure 1. Cell proliferation of U2OS cells after stimulated with PTH.

- (A) U2OS cells cultured in DMEM, stimulated with 1ng/mL PTH for 48h;
- (B) Unstimulated U2OS cells cultured in DMEM for 48h.

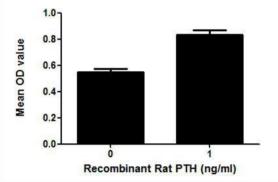


Figure 2. Cell proliferation of U2OS cells after stimulated with PTH.



### [IDENTIFICATION]

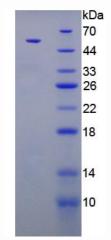


Figure 3. SDS-PAGE

Sample: Active recombinant PTH, Rat

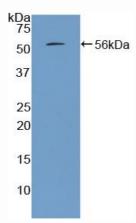


Figure 4. Western Blot Sample: Recombinant PTH, Rat; Antibody: Rabbit Anti-Rat PTH Ab (PAA866Ra01)