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APA441Hu02 100µg Active Luteinizing Hormone (LH) Organism Species: *Homo sapiens (Human) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Ala25~Ser116+GGGGS*3+Ser21~Leu141 Tags: N-terminal His-tag Purity: >90% Traits: Freeze-dried powder Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 8.0

Predicted Molecular Mass: 28.0kDa

Accurate Molecular Mass: 43kDa 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

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give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

[<u>USAGE</u>]

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]

APDVQD CPECTLQENP FFSQPGAPIL QCMGCCFSRA YPTPLRSKKT MLVQKNVTSE STCCVAKSYN RVTVMGGFKV ENHTACHCST CYYHKS GGGGSGGGGGGG SREPLRPWCH PINAILAVEK EGCPVCITVN TTICAGYCPT MMRVLQAVLP PLPQVVCTYR DVRFESIRLP GCPRGVDPVV SFPVALSCRC GPCRRSTSDC GGPKDHPLTC DHPQLSGLLF L

[ACTIVITY]

Luteinizing Hormone (LH) is a 42 kDa heterodimer belonging to the glycoprotein hormone family. It is composed of noncovalently linked glycosylated alpha and beta chains. The alpha subunit (CG alpha) is also a component of Follicle-Stimulating Hormone (FSH), Thyroid-Stimulating Hormone, and Chorionic Gonadotropin. The unique beta subunit confers the protein's specific biological action and is responsible for the interaction with its receptor.

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LH is produced and secreted by the anterior pituitary gland. Its secretion is controlled by Gonadotropin-Releasing Hormone from the hypothalamus; however, LH secretion can also be stimulated by estradiol. A functional binding ELISA assay was conducted to detect the interaction of recombinant human CGa/LHb and recombinant human Dopamine Receptor D1 (DRD1). Briefly, biotin-linked CGa/LHb were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to DRD1-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 μ l stop solution to the wells and read at 450 nm immediately. The binding activity of CGa/LHb and DRD1 was shown in Figure 1, the EC50 for this effect is 0.158 ug/mL.



Figure 1. The binding activity of recombinant human CGa/LHb and recombinant human

DRD1

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[IDENTIFICATION]



Figure 2. Gene Sequencing (extract)



Figure 3. SDS-PAGE

Sample: Active recombinant LH, 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.