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APA220Mu01 100µg Active Adrenomedullin (ADM) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Pro24~Ala171 Tags: N-terminal His-tag Purity: >90% 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: 10.1 Predicted Molecular Mass: 18.2kDa Accurate Molecular Mass: 22kDa as determined by SDS-PAGE reducing conditions.

[<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.

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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]

PDTPSQF RKKWNKWALS RGKRELQASS SYPTGLADET TVPTQTLDPF LDEQNTTGPL QASNQSEAHI RVKRYRQSMN QGSRSNGCRF GTCTFQKLAH QIYQLTDKDK DGMAPRNKIS PQGYGRRRRR SLLEVLRSRT VESSQEQTHT A

[ACTIVITY]

Adrenomedullin (ADM) is a peptide hormone first discovered in 1993 in pheochromocytoma. Although AM is highly expressed in the adrenal glands, heart, lungs, and kidneys, vascular endothelium and smooth muscle are thought to be the main source of plasma AM. It is a 52 aa peptide with several functions, including vasodilation, regulation of hormone secretion, promotion of angiogenesis, and antimicrobial activity. The antimicrobial activity is antibacterial, as the peptide has been shown to kill E. coli and S. aureus at low concentration. Receptor activity-modifying protein 2 can transports the calcitonin gene-related peptide type 1 receptor (CALCRL) to the plasma membrane. Acts as a receptor for adrenomedullin (AM) together with CALCRL. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse ADM and recombinant human RAMP2. Briefly, ADM was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to RAMP2-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ADM pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells

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were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50 µL stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant mouse ADM and recombinant human RAMP2 was shown in Figure 1, the EC50 for this effect is 0.16 ug/mL.



Figure 1. The binding activity of recombinant mouse ADM and recombinant human



RAMP2

Figure 2. Gene Sequencing (extract)

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kDa 70
44
33
26
22
18
14
10

Figure 3. SDS-PAGE

Sample: Active recombinant ADM, Mouse

[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.