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APA656Hu61 100µg Active Amiloride Binding Protein 1 (ABP1) 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: Glu20~Val751

Tags: N-terminal His-tag

Purity: >98%

Endotoxin Level: <1.0EU per $1\mu g$ (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, 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: 6.6

Predicted Molecular Mass: 85.1kDa

Accurate Molecular Mass: 100kDa 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.

[<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]

E PSPGTLPRKA GVFSDLSNQE LKAVHSFLWS KKELRLQPSS TTTMAKNTVF LIEMLLPKKY HVLRFLDKGE RHPVREARAV IFFGDOEHPN VTEFAVGPLP GPCYMRALSP RPGYOSSWAS RPISTAEYAL LYHTLQEATK PLHOFFLNTT GFSFODCHDR CLAFTDVAPR GVASGORRSW LIIORYVEGY FLHPTGLELL VDHGSTDAGH WAVEQVWYNG KFYGSPEELA RKYADGEVDV VVLEDPLPGG KGHDSTEEPP LFSSHKPRGD FPSPIHVSGP RLVOPHGPRF RLEGNAVLYG GWSFAFRLRS SSGLQVLNVH FGGERIAYEV SVQEAVALYG GHTPAGMQTK YLDVGWGLGS VTHELAPGID CPETATFLDT FHYYDADDPV HYPRALCLFE MPTGVPLRRH FNSNFKGGFN FYAGLKGQVL VLRTTSTVYN YDYIWDFIFY PNGVMEAKMH ATGYVHATFY TPEGLRHGTR LHTHLIGNIH THLVHYRVDL DVAGTKNSFO TLOMKLENIT NPWSPRHRVV OPTLEOTOYS WEROAAFRFK RKLPKYLLFT SPOENPWGHK RTYRLOIHSM ADOVLPPGWO EEQAITWARY PLAVTKYRES ELCSSSIYHO NDPWHPPVVF EQFLHNNENI ENEDLVAWVT VGFLHIPHSE DIPNTATPGN SVGFLLRPFN FFPEDPSLAS RDTVIVWPRD NGPNYVORWI PEDRDCSMPP PFSYNGTYRP V

[ACTIVITY]

Amiloride binding protein (ABP1), also known as amine oxidase copper domain-containing protein 1 (AOC1), is an approximately 110 kDa member of the copper-containing amine oxidase family. ABP1 is a glycosylated enzyme that forms disulfide-linked homodimers. It is stored in cytoplasmic granules of epithelial cells in the kidney, placenta, uterus, lung, and intestine, and its extracellular release can be induced by heparin. ABP1 exhibits a substrate preference for histamine and putrescine, generating hydrogen peroxide and ammonia in an oxidative deamination reaction. ABP1 activity plays an important role in the catabolism of histamine and other bioactive polyamines. The activity of recombinant human ABP1 was measured by its ability to produce hydrogen peroxide during the oxidation of histamine. The reaction was performed in 50 mM HEPES, pH 7.5 (assay buffer), initiated by addition 50 μ L of various concentrations of ABP1 (diluted by assay buffer) to 50 µl substrate mixture of 10 uM histamine, 2 units/mL HRP and 100 µM AUR. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively in kinetic mode for 5 minutes.

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RFU (544/590)	H ₂ O ₂ (product) uM
<u>539.79</u>	7.8125
275.79	3.90625
142.19	1.953125
73.98	0.9765625
37.9	0.48828125
17.96	0.244 140 62 5
10.31	0.122070313
4.57	0.061035156

Figure 1. The standard curve of H₂O₂

One unit of enzyme activity is defined as the 1 μ g of enzyme required to convert 1 pmol of histamine to H₂O₂ in 1min at 37°C. The specific activity of recombinant human ABP1/AOC1 is > 500 pmol/min/ μ g.

Specific Activity (pmol/min/µg)= $\frac{\Delta OD * F}{T * N}$

△OD=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of H₂O₂)

T= Time

N=Amount of enzyme

[IDENTIFICATION]





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Figure 3. SDS-PAGE

Sample: Active recombinant DAO, 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.