APC298Mu61 100µg Active Amine Oxidase, Copper Containing 3 (AOC3) 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: Eukaryotic expression. Host: 293F cell Residues: Arg28~Asn765 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 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: 6.3 Predicted Molecular Mass: 83.4kDa Accurate Molecular Mass: 85kDa 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.

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

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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.

[<u>SEQUENCE</u>]

RSGDGGGLSQPLHCPSVLPSVQPRTHPSQSQPFADLSPEELTAVMSFLTKHLGPGLVDAAQARPSDNCV FSVELQLPAKAAALAHLDRGGPPPVREALAIIFFGGQPKPNVSELVVGPLPHPSYMRDVTVERHGGPLP YYRRPVLDREYQDIEEMIFHRELPQASGLLHHCCFYKHQGQNLLTMTTAPRGLQSGDRATWFGLYYNLS GAGFYPHPIGLELLIDHKALDPALWTIQKVFYQGRYYESLTQLEDQFEAGLVNVVLVPNNGTGGSWSLK SSVPPGPAPPLQFHPQGPRFSVQGSQVSSSLWAFSFGLGAFSGPRIFDIRFQGERVAYEISVQEAIALY GGNSPASMSTCYVDGSFGIGKYSTPLIRGVDCPYLATYVDWHFLLESQAPKTLRDAFCVFEQNQGLPLR RHHSDFYSHYFGGVVGTVLVVRSVSTLLNYDYIWDMVFHPNGAIEVKFHATGYISSAFFFGAGEKFGNR VGAHTLGTVHTHSAHFKVDLDVAGLKNWAWAEDMAFVPTIVPWQPEYQMQRLQVTRKLLETEEEAAFPL GGATPRYLYLASNHSNKWGHRRGYRIQILSFAGKPLPQESPIEKAFTWGRYHLAVTQRKEEEPSSSSIF NQNDPWTPTVNFTDFISNETIAGEDLVAWVTAGFLHIPHAEDIPNTVTAGNSVGFFLRPYNFFDEDPSF HSADSIYFREGQDATACEVNPLACLSQTATCAPEIPAFSHGGFAYRDN

[ACTIVITY]

Amine Oxidase, Copper Containing 3 (AOC3) is a copper amine oxidase with a topaquinone cofactor. AOC3 is a Type II integral membrane protein, but a soluble form of the enzyme is present in human serum, and its level increases in diabetes and some inflammatory liver diseases. AOC3 catalyzes the oxidative deamination of small primary amines such as methylamine, benzylamine, and aminoacetone in a reaction that produces an aldehyde, ammonia, and H2O2. The enzyme is sensitive to inhibition by semicarbazide. AOC3 expression is highest in the endothelium of lung, heart, and intestine, but low in tissues such as brain, spleen, kidney, and liver. The activity of recombinant mouse AOC3 was measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The reaction was performed in 50 mM HEPES, pH 7.5 (assay buffer), initiated by addition 50 μ L of various concentrations of AOC3 (diluted by assay buffer) to 50 μ l substrate mixture of 2 mM Benzylamine, 2 units/mL HRP and 100 μ M 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
922.91	15.625
481.51	7.8125
272.81	3.90625
138.01	1.953125
69.05	0.9765625
33.86	0.48828125
15.68	0.244140625
6.82	0.122070313
2.55	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 benzylamine to H₂O₂ in 1min at 37°C. The specific activity of recombinant mouse AOC3 is > 6 pmol/min/ μ g.

Specific Activity (pmol/min/µg)= $\frac{\Delta OD * F}{T * N}$ $\triangle OD$ =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 2. SDS-PAGE Sample: Active recombinant AOC3, 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.