Recombinant Mouse VAP-1/AOC3

Catalog Number: 6107-AO

DESCRIPTION

Source *Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived

Met	10-His tag	GGGSGGGSGGGS	IEGR	Mouse VAP-1/AOC3 (Gly27-Asn765) Accession # O70423

N-terminus C-terminus

N-terminal Sequence Analysis Met

Structure / Form Disulfide-linked homodimer

Predicted Molecular Mass 85 kDa

SPECIFICATIONS

SDS-PAGE 80-90 kDa, reducing conditions

Activity Measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The specific activity is >10 pmol/min/µg, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, pH 7.5
- Recombinant Mouse VAP-1/AOC3 (rmVAP-1) (Catalog # 6107-AO)
- Coupling Agent: Horseradish Peroxidase (HRP) (250-330 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: Benzylamine (Sigma, Catalog # B5136), 100 mM stock in deionized water
- Substrate Component 2: Amplex Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmVAP-1 to 8 ng/µL in Assay Buffer.
2. Prepare the Substrate mixture 2 mM Benzylamine, 2 units/mL HRP and 100 µM AUR in Assay Buffer.
3. In a plate, load 50 µL of 8 ng/µL rmVAP-1 and start the reaction by adding 50 µL of the Substrate mixture (step 2). Include a Substrate Blank containing 50 µL of the Assay Buffer and 50 µL of the Substrate mixture.
4. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively in kinetic mode for 5 minutes. Note: A manual cutoff must be set at a wavelength of 570 nm.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using a fluorescent standard prepared by incubating 50 µM AUR, 1 unit/mL HRP, 1 mM Benzylamine, and a curve of Hydrogen Peroxide (Sigma, Catalog # H1009) in Assay Buffer. Use this oxidized AUR curve to determine the conversion factor.

Final Assay Conditions Per Well:

- rmVAP-1: 0.4 µg
- Benzylamine: 1 mM
- HRP: 1 unit/mL
- AUR: 50 µM

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Vascular adhesion protein-1 (VAP-1) is a copper amine oxidase with a topaquinone co-factor. VAP-1 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 (1, 2). VAP-1 catalyzes the oxidative deamination of small primary amines such as methylamine, benzylamine, and aminoacetone in a reaction that produces an aldehyde, ammonia, and H_2O_2 (3). The enzyme is sensitive to inhibition by semicarbazide. VAP-1 expression is highest in the endothelium of lung, heart, and intestine, but low in tissues such as brain, spleen, kidney, and liver (4). VAP-1 vascular expression is regulated at sites of inflammation through its release from intracellular granules in which the protein is stored (5). The adhesive function of VAP-1 has been demonstrated in studies showing that the protein is important for the adherence of certain lymphocyte subtypes to inflamed endothelial tissues (6). VAP-1 mediated adhesion is involved in the process of leukocyte extravasation, an important feature of inflammatory responses. The role of VAP-1 amine oxidase activity in this process is not fully defined, but it appears to be carbohydrate-dependent (7). VAP-1 is considered to be a therapeutic target for diabetes, oxidative stress, and inflammatory diseases (8). The N-terminal transmembrane domain of recombinant mouse VAP-1 was deleted and replaced with a signal sequence, resulting in the secretion of the soluble form of the protein.

References:

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