

**DESCRIPTION**

<b>Source</b>	<i>Spodoptera frugiperda</i> , Sf 21 (baculovirus)-derived Gly27-Asn763, with an N-terminal Met and 10-His tag, followed by a Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Ile-Glu-Gly-Arg linker Accession # Q16853
<b>N-terminal Sequence Analysis</b>	Met
<b>Structure / Form</b>	Disulfide-linked homodimer
<b>Predicted Molecular Mass</b>	85 kDa

**SPECIFICATIONS**

<b>SDS-PAGE</b>	80-90 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The specific activity is >8 pmol/min/μg, as measured under the described conditions.
<b>Endotoxin Level</b>	<1.0 EU per 1 μg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
<b>Formulation</b>	Supplied as a 0.2 μm filtered solution in HEPES and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

<b>Materials</b>	<ul style="list-style-type: none"> <li>● Assay Buffer: 50 mM HEPES, pH 7.5</li> <li>● Recombinant Human VAP-1/AOC3 (rhVAP-1) (Catalog # 3957-AO)</li> <li>● Coupling Enzyme: Horseradish Peroxidase (HRP) (250 - 330 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0</li> <li>● Substrate Component 1: Benzylamine (Sigma, Catalog # B5136), 100 mM stock in deionized water</li> <li>● Substrate Component 2: Amplex® Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 10 mM stock in DMSO</li> <li>● F16 Black Maxisorp Plate (Nunc, Catalog # 475515)</li> <li>● Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent</li> </ul>
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<b>Assay</b>	<ol style="list-style-type: none"> <li>1. Dilute rhVAP-1 to 20 ng/μL in Assay Buffer.</li> <li>2. Prepare the Substrate mixture 2 mM Benzylamine, 2 units/mL HRP and 100 μM AUR in Assay Buffer.</li> <li>3. In a plate, load 50 μL of 20 ng/μL rhVAP-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.</li> <li>4. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively in kinetic mode for 5 minutes. Note: A cutoff must be set at a wavelength of 570 nm.</li> <li>5. Calculate specific activity:</li> </ol>
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$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$$

\*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.

<b>Final Assay Conditions</b>	<p>Per Well:</p> <ul style="list-style-type: none"> <li>● rhVAP-1: 1.0 μg</li> <li>● Benzylamine: 1 mM</li> <li>● HRP: 1 unit/mL</li> <li>● AUR: 50 μM</li> </ul>
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**PREPARATION AND STORAGE**

<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>● 6 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 3 months, -20 to -70 °C under sterile conditions after opening.</li> </ul>

**BACKGROUND**

Vascular adhesion protein-1 (VAP-1) is a copper amine oxidase with a topaquinone cofactor. 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<sub>2</sub>O<sub>2</sub> (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).

**References:**

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