Recombinant Human Vanin-1/VNN1

Catalog Number: 7999-AH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Gln22-Ser490, with a C-terminal 6-His tag Accession # O95497
N-terminal Sequence Analysis	No results obtained: Gln22 predicted
Predicted Molecular Mass	53 kDa

SPECIFICATIONS

SDS-PAGE	60-80 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze pantetheine to pantothenate and cysteamine. The specific activity is >1500 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, 2 mM DTT, 1% Brij-35 (w/v), pH 7.0
- Recombinant Human Vanin-1/VNN1 (rhVNN1) (Catalog # 7999-AH)
- Substrate: Pantethine (Sigma, Catalog # P2125) 50 mM stock in deionized water
- o-pthalaldehyde (Sigma, Catalog # P0657) 50 mg/mL (373 mM) stock in DMSO
- 0.5 M Sodium Borate, pH 9.0
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhVNN1 to 1 µg/mL in Assay Buffer.
2. Dilute Substrate to 500 µM in Assay Buffer.
3. Load 50 µL of dilute rhVNN1 to empty wells of a black well plate.
4. Start reaction by adding 50 µL of dilute substrate to wells containing enzyme. Create Enzyme Controls by not adding substrate to the wells.
5. Seal plate with a plate sealer and incubate at 37 °C for 30 minutes.
6. Prepare Detection mixture containing 15 mM oPA in 0.5 M sodium borate, pH 9.0.
7. Add 100 µL Detection mixture to all wells used. For Enzyme Controls, add dilute substrate after Detection Mixture is added.
8. Mix well and incubate sealed plate at room temperature for 5 minutes.
9. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
10. Calculate specific activity:

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

*Adjusted for Enzyme Control

**Derived using calibration standard Cysteamine Hydrochloride (Sigma, Catalog # M6500).

Final Assay Conditions

Per Well:

- rhVNN1: 0.050 µg
- Substrate: 125 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.

BACKGROUND

Vanin-1 (VNN1) is a member of the biotinidase family and is expressed at the cell surface in epithelial cells (1). VNN1 is also known as vascular non-inflammatory molecule 1. It does not possess biotinidase activity, but is a pantetheinase that catalyzes the hydrolysis of pantetheine to pantothenic acid (vitamin-B5) and cysteamine (2, 3). VNN1 is considered to be a potential biomarker for the acute kidney injury (4) and a target for therapeutic intervention in inflammatory bowel disease (5). Recombinant human VNN1 was engineered to have a C-terminal truncation that prevents the normal GPI-anchor modification, resulting in its secretion.

References:

1. Pitari, G. *et al.* (2000) FEBS Lett. **483:**149.
2. Maras, B. *et al.* (1999) FEBS Lett. **461:**149.
3. Martin, F. *et al.* (2001) Immunogenetics **53:**296.
4. Hosohata, K. *et al.* (2012) J. Phamacol. Exp. Ther. **341:**656.
5. Berruyer, C. *et al.* (2006) J. Exp. Med. **203:**2817.

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