Recombinant Human DPP3

Catalog Number: 8087-SE

DESCRIPTION

Source	*E. coli*-derived Ala2-Ala737, with an N-terminal Met and 6-His tag Accession # Q9NY33
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	83 kDa

SPECIFICATIONS

SDS-PAGE	68-75 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Arg-Arg-7-amido-4-methylcoumarin (RR-AMC). The specific activity is >2,300 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	>90%, 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 MES, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 150 mM NaCl, 0.02% (w/v) Brij-35, pH 9.0
- Recombinant Human DPP-3 (rhDPP-3) (Catalog # 8087-SE)
- Substrate: H-Arg-Arg-AMC (Bachem, Catalog # I-1055), 10 mM stock in deionized water.
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhDPP-3 to 0.1 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load 50 µL of 0.1 ng/µL rhDPP-3 into a plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate.
4. Read at excitation and emission wavelengths of 345 nm and 445 nm, respectively in kinetic mode for 5 minutes.
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 calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

Final Assay Conditions

Per Well:

- rhDPP-3: 0.005 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

DPP3 (DiPeptidyl Peptidase III; also known as Dipeptidyl arylamidase III and Enkephalinase B) is a cytosolic member of the metallopeptidase family of proteins (1, 2). More specifically, it is classified as the singular member of the M49/Clan M- family of enzymes that possesses an unusual six-amino acid, zinc-binding motif (HExxGH) (1, 3). Notably, DPP # 3 is the only DPP that qualifies as a metallopeptidase, as all other DPPs belong to either the cysteine or serine class of peptidases. DPP3 is widely expressed, being found in numerous hematopoietic cells (RBCs, neutrophils, monocytes) and epithelium-dominated tissues (1, 4, 5). Although DPP3 was initially reported to be an Arg-Arg dipeptidase for non-N-terminally substituted peptides, it is now known to be active on a wide range of amino acid combinations, and thus qualifies as a non-specific peptidase. DPP3 does show restriction when it comes to peptide length, however; peptides shorter than three and longer than ten amino acids are very poor substrates for DPP3. Consistent with its broad range of substrates, DPP3 likely has multiple functions. It has been suggested to be a general mediator of peptidome degradation (i.e. the three-to-24 amino acid cytoplasmic fragments that result from initial proteasome degradation), and is considered particularly important in the degradation of proline-containing peptides (1, 6). Conversely, elevated levels of DDP3 activity will reduce the availability of eight-to-ten amino acid length peptides that are used for MHC presentation, adversely affect this crucial immune surveillance activity. DDP3 is also found extracellularly, and has documented activity against angiotensin II-IV and opioids, suggesting a role for DPP3 in both blood pressure regulation and pain modulation (1, 6-8). Finally, DPP3 appears to play a protective role in oxidative stress. Nrf2 is a Zn-finger transcription factor that stimulates antioxidant enzyme production. Normally, it is sequestered in the cytosol through complex formation with Keap I. Though the details are somewhat unclear, under oxidative stress, DPP3 appears to promote the dissociation of Nrf2 and Keap I, directing Nrf2 into the nucleus with subsequent antioxidant enzyme transcription (1). Human DPP3 is 737 amino acids in length, contains a peptidase region over aa 143-705, and is reported to run as a 93-94 kDa protein on SDS-PAGE (2). Although there is no canonical signal sequence, as noted, it is found extracellularly. Potential sites for myristoylation are known and, if utilized, may account for reports of a DPP3 presence in membranes (1). There are potential isoform variants. One shows a deletion of aa 182-601, while another shows a deletion of aa 91-120. Mouse and rat DPP3 share 93% aa sequence identity with human DPP3.

References:

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