

Background

Dipeptidyl-peptidase II (DPPII) is identical to quiescent cell proline dipeptidase (QPP) and dipeptidylpeptidase 7 (DPP7) (1, 2). It shares some substrate and cleavage specificity with DPPIV/CD26, DPP8, DPP9 and seprase/FAP (fibroblast activation protein), members of the S09 family of serine proteases. As prolyl proteases that cleave proteins and peptides after proline residues, these enzymes have high potential for drug discovery (3, 4). However, DPP7 is not a member of the S09 family, but a member of the S28 family that also includes lysosomal Pro-X carboxypeptidase/prolylcarboxypeptidase/PRCP and thymus-specific serine peptidase/PRSS16 (2). The human DPP7 precursor consists of a signal peptide (aa 1 - 21) and a mature chain (aa 22 - 492). The purified rhDPP7 is active against Lys-Pro-AMC and Lys-Ala-AMC. Its activity against Lys-Pro-AMC is approximately 10-fold of that against Lys-Ala-AMC under otherwise identical conditions.

References:

1. Underwood, R. *et al.* (1999) *J. Biol. Chem.* **274**:34053.
2. Maes, M.B. *et al.* (2005) *Biochem. J.* **386**:315.
3. Rosenblum, J.S. and J.W. Kozarich (2003) *Curr. Opin. Chem. Biol.* **7**:496.
4. Lankas, G.R. *et al.* (2005) *Diabetes* **54**:2988.

Description

Source	Murine myeloma cell line, NS0-derived Gly22 - Leu492, with a C-terminal 10-His tag Accession # AAH11907
N-terminal Sequence Analysis	Gly22 & Arg24
Predicted Molecular Mass	54 kDa

Specifications

SDS-PAGE	64 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Lys-Pro-AMC (KP-AMC). The specific activity is > 4,000 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

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. <ul style="list-style-type: none"> • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM MES, pH 6.0
- Recombinant human DPP7 (R&D Systems, Catalog # 3438-SE)
- Substrate Lys-Pro-AMC (Bachem, Catalog # I-1745), 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 rhDPP7 to 0.2 ng/μL in Assay Buffer.
2. Dilute Substrate to 200 μM in Assay Buffer.
3. Load into a black microplate 50 μL of 0.2 ng/μL rhDPP7, and start the reaction by adding 50 μL of 200 μM Substrate. Include a Substrate Blank containing Assay Buffer in place of rhDPP7 and Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmole/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

Final Assay Conditions Per Well

- rhDPP7: 0.01 μg
- Substrate: 100 μM

10/19/2009

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