Recombinant Human DPPIV/CD26 (High Purity Dimer)

Catalog Number: 9168-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Asn29-Pro766, with a C-terminal Asp-Ile and 6-His tag Accession # CAA43118
N-terminal Sequence Analysis	Asn29
Structure / Form	Noncovalently-linked homodimer
Predicted Molecular Mass	86 kDa

SPECIFICATIONS

SDS-PAGE	95-110 kDa, reducing condtions
Size Exclusion Chromatography	5% aggregates and 12% monomer
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Gly-Pro-7-amido-4-methylcoumarin (GP-AMC). The specific activity is >3,900 pmol/min/µg as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, pH 8.0
- Recombinant Human DPPIV/CD26 (rhDPPIV) (Catalog # 9168-SE)
- Substrate: H-Gly-Pro-AMC (Bachem, Catalog # I-1225), 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 rhDPPIV to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load into a black plate 50 µL of 0.2 ng/µL rhDPPIV, and start the reaction by adding 50 µL of 20 µM Substrate. As a Substrate Blank combine 50 µL of Assay Buffer and 50 µL of 20 µM 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:

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

*Adjusted for Substrate Blank.
**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A9891).

Final Assay Conditions

Per Well:

- rhDPPIV: 0.010 µg
- Substrate: 10 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

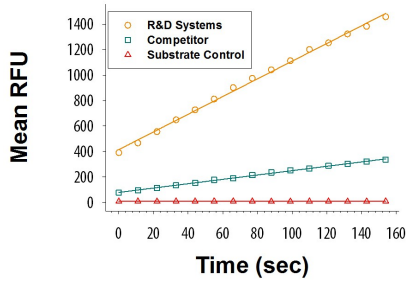
bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

Recombinant Human DPPIV/CD26 (High Purity Dimer)

Catalog Number: 9168-SE

DATA

Enzyme Activity



Recombinant Human DPPIV/CD26 (Catalog # 9168-SE) is measured by its ability to cleave the fluorogenic peptide substrate, Gly-Pro-7-amido-4-methylcoumarin (GP-AMC). The activity (orange) is approximately 4-fold greater than the competitor's DPPIV/CD26 (green).

BACKGROUND

DPPIV/CD26 (EC 3.4.14.5) is an approximately 110 kDa serine exopeptidase that releases Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of oligo- and polypeptides. It regulates immune and endocrine function through the cleavage of multiple chemokines, growth factors, and peptide hormones (1, 2). Mature human DPPIV consists of a 6 amino acid (aa) cytoplasmic tail, a 22 aa transmembrane segment, and a 738 aa extracellular domain (ECD) that contains the catalytic active site (Ser, Asp, and His charge relay system) (3). Within the ECD, human DPPIV/CD26 shares 84% amino acid sequence identity with mouse and rat DPPIV. DPPIV is expressed as a noncovalent homodimer on the surface of epithelial cells, endothelial cells, and activated lymphocytes, and it can be released by MMP mediated shedding (4). It cleaves a range of peptide hormones including Glucagon, Glucagon-like Peptides 1 and 2, GIP, GHRH, Procalcitonin, Neuropeptide Y, and Substance P (5). It is released from adipocytes and induces insulin resistance in adipocytes and skeletal muscle (6). DPPIV also cleaves many chemokines, resulting in reduced chemotactic activity of CXCL6, 9, 10, 11, 12, and CCL5 (7-10) but unchanged angiostatic activity of CXCL9 and CXCL10 (8). Cleavage can increase (CCL5), decrease (CXCL12), or have no effect (CCL4) on chemokine blockade of HIV-1 cellular infectivity (7, 9, 11). In addition, DPPIV cleavage of CCL4 broadens chemokine receptor usage to also include CCR2b (11). DPPIV serves as a cell entry coreceptor for HIV and coronavirus (12, 13). It cleaves human GM-CSF and IL-3 and reduces their ability to promote myeloid cell development (14). It also interferes with CXCL12 induced hematopoietic cell migration, homing, and engraftment (15). DPPIV interacts in cis with adenosine deaminase on T cells and in trans with caveolin-1 on antigen presenting cells (16, 17). It provides costimulatory proliferation and activation signals to both $CD4^+$ and CD8+ T cells (17, 18).

References:

1. Klemann, C. *et al.* (2016) Clin. Exp. Immunol. Epub PMID 26919392.
2. Mortier, A. *et al.* (2016) J. Leukoc. Biol. Epub PMID 26744452.
3. Tanaka, T. *et al.* (1992) J. Immunol. **149**:481.
4. Rohrborn, D. *et al.* (2014) FEBS Lett. **588**:3870.
5. Waumans, Y. *et al.* (2015) Front. Immunol. **6**:387.
6. Lamers, D. *et al.* (2011) Diabetes **60**:1917.
7. Proost, P. *et al.* (1998) J. Biol. Chem. **273**:7222.
8. Proost, P. *et al.* (2001) Blood **98**:3554.
9. Ohtsuki, T. *et al.* (1998) FEBS Lett. **431**:236.
10. Barreira da Silva, R. *et al.* (2015) Nat. Immunol. **16**:850.
11. Guan, E. *et al.* (2002) J. Biol. Chem. **277**:32348.
12. Callebaut, C. *et al.* (1993) Science **262**:2045.
13. Raj, V.S. *et al.* (2013) Nature **495**:251.
14. Broxmeyer, H.E. *et al.* (2012) Nat. Med. **18**:1786.
15. Christopherson II, K.W. *et al.* (2004) Science **305**:1000.
16. Kameoka, J. *et al.* (1993) Science **261**:466.
17. Ohnuma, K. *et al.* (2007) J. Biol. Chem. **282**:10117.
18. Hatano, R. *et al.* (2013) Immunology **138**:165.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956