Recombinant Cynomolgus Monkey DPPIV/CD26

Catalog Number: 9637-SE

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Asn29-Pro766 with a C-terminal 6-His tag Accession # XP_005573374
N-terminal Sequence Analysis	Asn29
Predicted Molecular Mass	86 kDa

SPECIFICATIONS

SDS-PAGE	100-116 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Gly-Pro-7-amido-4-methylcoumarin (GP-AMC). The specific activity is >3,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, pH 8.0
- Recombinant Cynomolgus Monkey DPPIV/CD26 (rcynoDPPIV) (Catalog # 9637-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 rcynoDPPIV to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of 0.2 ng/µL rcynoDPPIV into a plate, and start the reaction by adding 50 µL of 20 µM Substrate. For Substrate Blank, load 50 µL of Assay Buffer and 50 µL of Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), 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:

- rcynoDPPIV: 0.010 µg
- Substrate: 10 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

DPPIV/CD26 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 DPPIV consists of a cytoplasmic tail, a transmembrane segment, and an extracellular domain (ECD) that contains the catalytic active site (Ser, Asp, and His charge relay system) (3). Within the ECD, cynoDPPIV/CD26 shares 96% amino acid sequence identity with human DPPIV. DPPIV is expressed as a nocovalent 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. **185**:1.
2. Metzemaekers, M. *et al.* (2016) Front Immunol. **7**:483.
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.

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