Recombinant Human ADAM9 His-tag

Catalog Number: 939-ADB

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human ADAM9 protein Ala206-Asp697, with a C-terminal 10-His tag Accession # Q13443.1
N-terminal Sequence Analysis	Ala206
Structure / Form	Mature form
Predicted Molecular Mass	55 kDa

SPECIFICATIONS

SDS-PAGE	61-68 kDa, under reducing conditions.
Activity	Measured by its ability to cleave a fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH_2 (Catalog # ES003). The specific activity is >5 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 2.5 µM $ZnCl_2$, 0.005% (w/v) Brij-35, pH 9.0
- Recombinant Human ADAM9 His-tag (rhADAM9) (Catalog # 939-ADB)
- Fluorogenic Peptide Substrate III: MCA-Pro-Leu-Ala-Gln-Ala-Val-DPA-Arg-Ser-Ser-Ser-Arg-NH3 (Catalog # ES003)
- 96-Well Black Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhADAM9 to 40 µg/mL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Combine equal volumes of 40 µg/mL rhADAM9 and 200 µM Substrate. Include a Substrate Blank by combining equal volumes of Assay Buffer and 20 µM Substrate.
4. Incubate the Reactions and Substrate Blank at 37 °C for 30 minutes.
5. Load 100 µL of each incubated Reaction and Substrate Blank into wells of a black plate.
6. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in endpoint mode.
7. 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 Substrate Blank
**Derived using calibration standard MCA-Pro-Leu-OH.

Final Assay Conditions

Per Well:

- rhADAM9: 2 µg
- Substrate: 100 µM

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

Recombinant Human ADAM9 His-tag

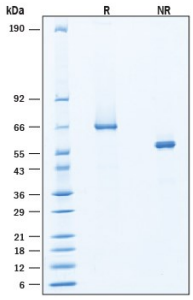
Catalog Number: 939-ADB

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.

DATA

SDS-PAGE



Recombinant Human ADAM9 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human ADAM9 His-tag Protein (Catalog # 939-ADB) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 61-68 kDa, under reducing conditions.

BACKGROUND

Recombinant human ADAM9, also known as MDC9 or meltrin gamma, is a member of the ADAM family that contains "a disintegrin and metalloprotease like" domain (1,2). ADAM9 is widely expressed on the cell surface across multiple cell types and tissues where it is involved in diverse biological functions in the extracellular matrix (ECM) (2). Like other membrane-anchored ADAMs, expressed ADAM9 has a C-shaped structure that facilitates substrate recognition and cleavage and consists of a prodomain with a cysteine switch and furin cleavage sequence, a catalytic domain with the zinc binding site, along with several additional domains: a disintegrin-like domain, a cysteine-rich domain, an EGF-like domain, a transmembrane domain, and the cytoplasmic domain (2). A zinc ion in the catalytic domain of ADAM-9 coordinates with the prodomain to maintain latency. Removal of the propeptide through cleavage by proprotein convertases activates the protein (2). ADAM9 has specificity for targets containing an established preferred consensus sequence including a broad range of substrates within the ECM such as tumor necrosis factor-alpha (TNF-alpha), growth factors and their receptors such as the p75 TNF receptor, and key molecules beta -amyloid protein precursor, c kit ligand-1, oxidized insulin beta -chain, and fibronectin (2-4). In addition to catalytic activity, ADAM9 functions as an adhesion molecule through binding of its disintegrin domain (2,5,6) and the cytoplasmic domain of ADAM9 interacts with Src homology 3 (SH3) containing proteins and protein kinase C (2,7,8). Given the broad expression and diverse activity of ADAM9, it plays an important role in pathological diseases including degenerative diseases such as Alzheimers, inflammatory diseases such as COPD and tumor biology in many cancers including lung, prostate, liver, breast, pancreatic making it attractive as a biomarker and therapeutic target (2, 9-15).

References:

1. Moss, M.L. *et al.* (2001) Drug Discov. Today **6**:417.
2. Chou, C.W, *et al.* (2020) Int. J. Mol. Sci. **21**:7790.
3. Roghan, M. *et al.* (1999) J. Biol. Chem. **274**:3531.
4. Schwettmann, L. and H. Tschesche (2001) Prot. Express. & Purif. **21**:65.
5. Nath, D. *et al.* (2000) J. Cell Sci. **113**:2319.
6. Zhou, M. *et al.* (2001) Biochem. Biophys. Res. Comm. **280**:574.
7. Izumi, Y. *et al.* (1998) EMBO J. **17**:7260.
8. Howard, L. *et al.* (1999) J. Biol. Chem. 274:31693.
9. Cong, L. and J. Jia (2011) Neurobiol. Aging **32**:54.
10. Zhang, J. *et al.* (2014) J. Cancer Res. Clin. Oncol. **140**:1441.
11. Hua, Y. *et al.* (2018) Oncol. Lett. **15**:9051.
12. Liu, R. *et al.* (2018) Onco. Targets Ther. **11**:4065.
13. Wang, X. *et al.* (2018) Am. J. Respir. Crit. Care Med. **198**:1500.
14. Xing, C. *et al.* (2019) Artif. Cells Nanomed. Biotechnol. **47**:3920.
15. Oh, S. *et al.* (2020) Cancers **12**:745.

Bio-Techne®
Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956
USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475