

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived human ADAM12 protein
Arg29-Ser513, with an N-terminal signal peptide and a C-terminal 6-His tag
Accession # AAC08702

N-terminal Sequence Analysis Arg29

Structure / Form Pro & Mature forms

Predicted Molecular Mass 55 kDa (Pro) & 34 kDa (Mature)

SPECIFICATIONS

SDS-PAGE 25-30 kDa, 50-55 kDa & 68-72 kDa, reducing conditions

Activity Measured by its ability to cleave IGFBP-3.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 µm filtered solution in Sodium Acetate, CaCl₂ and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Activation Buffer: 50 mM Tris, 1 mM CaCl₂, 0.5% Brij-35, pH 9.0
 - Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, pH 7.5
 - Recombinant Human ADAM12 (rhADAM12) (Catalog # 4416-AD)
 - Recombinant Human Furin (rhFurin) (Catalog # 1503-SE)
 - Recombinant Human IGFBP-3 (rhIGFBP-3) (Catalog # 675-B3)
 - SDS-PAGE and/or Western blot

- Assay**
1. Dilute rhADAM12 to 200 µg/mL in Activation Buffer.
 2. Dilute rhFurin to 10 µg/mL in Activation Buffer.
 3. Mix 25 µL of rhADAM12 and 25 µL of rhFurin in a reaction tube.
 4. Incubate the mixture for 1 hour at 37 °C.
 5. Reconstitute or dilute rhIGFBP-3 to 200 µg/mL in Assay Buffer (do not follow reconstitution directions given by the 675-B3 product insert).
 6. Add 25 µL of rhIGFBP-3 dilution, 10 µL of activated rhADAM12, and 15 µL of Assay Buffer to a tube.
 7. Incubate reaction at 37 °C for 16 hours.
 8. As controls combine 25 µL of rhIGFBP-3 solution and 25 µL of Assay Buffer in each of two tubes. Incubate one tube at 37 °C and the other at -20 °C.
 9. Stop the reaction by adding SDS-PAGE sample buffer.
 10. Analyze the cleavage by SDS-PAGE followed by protein staining and/or Western blot.

- Final Assay Conditions**
- Per Reaction:
- rhADAM12: 20 µg/mL
 - rhIGFBP-3: 100 µg/mL

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.

BACKGROUND

ADAM12, also known as meltrin- α , is a member of the ADAM family with metalloprotease activity (1). It consists of a propeptide, metalloproteinase, disintegrin, cysteine-rich, and EGF-like domains, a transmembrane segment, and a cytoplasmic tail with SH3 binding motifs. Human ADAM12 exists in two alternatively spliced forms: the prototype transmembrane form and a shorter secreted form lacking the transmembrane domain and the cytoplasmic tail. The secreted form has a 34 amino acid substitution in place of the transmembrane and cytoplasmic regions. In mouse, only the transmembrane form has been observed. The propeptide, which is cleaved in the Golgi by furin-like proprotein convertases, is retained in a noncovalent complex after ADAM12 secretion (2). Thus, the pro domain may function as an inhibitor of the proteolytic activity or play another unknown function. The known physiological substrates of ADAM12 are HBEGF in the heart (3) and IGFBP-3 and -5 in placental serum (4). Its proteolytic activity is inhibited by the tissue inhibitor of metalloproteinase-3 (Recombinant Human TIMP-3, (Catalog # 973-TM) and α -2-macroglobulin. It also mediates cell-cell adhesion by interacting with integrins and syndecans as well as with additional unidentified molecules (4). ADAM12 may be a promising marker in prenatal diagnostics and breast cancer (5, 6). The recombinant ADAM12 contains the pro, metalloproteinase, and disintegrin domains. In addition to TIMP-3, the activity can also be inhibited by 5 mM 1,10-phenanthroline.

References:

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3. Asakura, M. *et al.* (2002) *Nat. Med.* **8**:35.
4. Loechel, F. *et al.* (2000) *Biochem. Biophys. Res. Comm.* **278**:511.
5. Laigaard, J. *et al.* (2006) *Prenat Diagn.* **26**:973.
6. Roopali, R. *et al.* (2004) *J. Biol. Chem.* **279**:51323.