

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Glu82-Asp1214 and Arg84-Asp1214, both with a C-terminal 10-His tag
Accession # Q13219

N-terminal Sequence Analysis Glu82 & Arg84

Structure / Form Disulfide-linked homodimer

Predicted Molecular Mass 128 kDa

SPECIFICATIONS

SDS-PAGE 115 kDa and 150 kDa, reducing conditions

Activity Measured by its ability to cleave IGFBP-5.
Cleavage of Recombinant Human IGFBP-5 (Catalog # 875-B5) by Recombinant Human Pappalysin-1/PAPP-A is >50%, as measured under the described conditions.
The IGFBP-5 cleaving activity of rhPAPP-A can be inhibited by 10 mM 1,10-phenanthroline.

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

Purity >90%, by SDS-PAGE under reducing conditions and visualized by silver stain.

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

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, pH 7.5 (TCN)
- Recombinant Human Pappalysin-1/PAPP-A (rhPappalysin-1) (Catalog # 2487-ZN)
- Recombinant Human IGFBP-5 (rhIGFBP-5) (Catalog # 875-B5)
- Reducing SDS-PAGE gel buffer
- SDS-PAGE and/or Western blot

Assay

1. Dilute rhPappalysin-1 to 92.8 µg/mL in Assay Buffer.
2. Dilute rhIGFBP-5 to 200 µg/mL in Assay Buffer.
3. Combine equal volumes of 92.8 µg/mL rhPappalysin-1 and 200 µg/mL rhIGFBP-5. Include two controls containing Assay Buffer in place of rhPappalysin-1.
4. Incubate reaction vials and one control at 37 °C for 30 minutes. Keep the other control at -20 °C during the 30 minute incubation period.
5. Combine equal volumes of rhPappalysin-1/rhIGFBP-5 reaction mixture and reducing SDS-PAGE gel buffer.
6. Analyze the cleavage by SDS-PAGE (load 20 µL/lane) followed by protein staining and/or Western blot.

Final Assay Conditions

Per Lane:

- rhPappalysin-1: 0.464 µg
- rhIGFBP-5: 1 µg

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

Pappalysins belong to a fifth family of metzincins that consists of ADAMs/ADAMTSs, MMPs, astacins and serrylsins (1). PAPP-A is an important pregnancy protein and increases in plasma by a factor of about 150 during pregnancy as compared to the nonpregnant state. PAPP-A is also a major marker of Down syndrome in the first trimester of pregnancy because maternal serum levels of PAPP-A are significantly reduced when a fetus affected by Down syndrome is present (2). PAPP-A cleaves Insulin-like Growth Factor-Binding Protein-4 and -5 (IGFBP-4 and -5) at a single site, resulting in the release of bioactive IGF (3). Lack of IGFBP-4 cleavage in embryonic fibroblasts derived from PAPP-A knockout mice indicates that PAPP-A functions as a physiological IGFBP-4 protease (4). Three Lin12-Notch repeats (LNR) in the PAPP-A protein bind Ca²⁺ and are required for the cleavage of IGFBP-4, not IGFBP-5, by PAPP-A (5). The C-terminal LNR (residues 1476 to 1503) is not present in rhPAPP-A (residues 82 to 1214), which starts at the N-terminus of the mature chain and ends before the five Sushi (SCR) modules. As an active protease, rhPAPP-A cleaves IGFBP-5, which can be inhibited by 1,10-phenanthroline.

References:

1. Boldt, H.B. *et al.* (2001) *Biochem. J.* **358**:359.
2. Fialova L. and I.M. Malbohan (2002) *Bratisl. Lek. Listy* **103**:194.
3. Laursen, L.S. *et al.* (2001) *FEBS Lett.* **504**:36.
4. Conover, C.A. *et al.* (2004) *Development* **131**:1187.
5. Boldt, H.B. *et al.* (2004) *J. Biol. Chem.* **279**:38525.