

MD Biosciences

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Enzyme

Recombinant Human Procollagenase-3 His-Tagged (EC 3.4.24)

Metalloproteinase 13 (MMP-13)

Product Code: 5028014

INTRODUCTION

Matrix metalloproteinases (MMP) are Zn^{2+} - and Ca^{2+} - dependent endopeptidases [1]. Main subfamilies of MMP are collagenases, gelatinases, stromelysins and membrane-type matrix metalloproteinases [2]. Three homologous collagenases have been identified in human tissues: interstitial collagenase, neutrophil collagenase and collagenase-3. The three enzymes cleave fibrillar collagens at a single site, generating fragments of approximately $\frac{1}{3}$ and $\frac{2}{3}$ the size of the original molecules.

Procollagenase-3 consists of 452 amino acids with a calculated M_r of 51.680 Da [3]. Due to N-linked glycosylation the apparent M_r is about 60.000 Da [4]. Within the protein the following domains and sequence regions can be distinguished [3, 4]: an N-terminal propeptide, which confers latency to the proenzyme, a Ca^{2+} - and Zn^{2+} -ions binding catalytic domain, a hinge region, and a C-terminal hemopexin-like domain.

Latent procollagenase-3 can be activated by proteases as stromelysin [4], gelatinase A, membrane-type 1 matrix metalloproteinase and plasmin [5] or by incubation with organomercurials (e.g. APMA) [4]. Active collagenase-3 begins with the N-terminal sequence YNVFPRTL [4].

Collagenase-3 hydrolyzes type II collagen 5- to 6-times faster than type I and type III collagens. The enzyme exhibits also high activity towards gelatin and it degrades SERPINS as α_1 -antichymotrypsin and plasminogen activator inhibitor-2 [4]. Collagenase-3 is inhibited in a 1:1 stoichiometric fashion by TIMP-1, TIMP-2 and TIMP-3.

Collagenase-3 is expressed during fetal bone development [6]. In adult human tissues collagenase-3 has been detected only in pathological conditions: in malignant tumors [3], in chronic ulcers [7], in arthritic cartilage [8] and synovium [9].

MOLECULARFORM

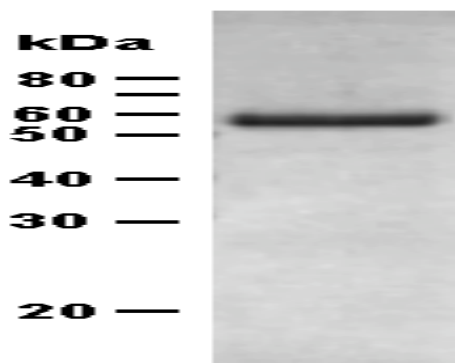
Human recombinant procollagenase-3 is expressed in Sf9-insect cells using the baculovirus expression vector system and purified from cell culture supernatant. The protein contains in addition to the 452 amino acids of full-length procollagenase-3 a C-terminal His-tag. The resulting M_r is 52.520 Da. Due to N-linked glycosylation the proenzyme appears as a band of about 60.000 Da in SDS-PAGE. Procollagenase-3 is solubilized in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 0.05 % Brij-35. Upon incubation with APMA active collagenase-3 with an apparent M_r of 48.000 Da is formed.

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PURITY

Recombinant procollagenase-3 appears as a single protein band of about 60.000 Da in SDS-PAGE (> 95 % of total protein). Due to autoproteolytic activity minor bands of activated collagenase-3 may be visible in the enzyme preparation.



SDS-PAGE of 1 μ g procollagenase-3

SPECIFIC ACTIVITY

The specific activity of activated collagenase-3 is \geq 200 mU/mg. 1 U is the activity that hydrolyzes 1 μ mol peptide (7-methoxycoumarin-4-yl) acetyl-Pro-Leu-Gly-Leu-(3-[2, 4-dinitrophenyl]-L-2, 3-diamino-propionyl)-Ala-Arg-NH₂ (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg) within 1 min under the assay conditions described below.

INHIBITORS

Collagenase-3 is inhibited by tissue inhibitors of matrix metalloproteinases (TIMP) and by chelators of divalent cations as EDTA or o-phenanthroline.

STORAGE

This product is stable until the expiry date given on the label when stored at -70 °C. The enzyme can be kept at -20 °C for several days. Repeated freezing and thawing should be avoided.

APPLICATONS

Recombinant procollagenase-3 can serve as antigen standard in immunochemical analyses. The active enzyme may be used to study the degradation of extracellular matrix proteins, to screen inhibitors of matrix metalloproteinases and to characterize inhibitor actions.

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Activation of procollagenase-3 and measurement of catalytic activity

Preparation and stability of solutions

APMA-solution: 40 mM p-aminophenyl mercuric acetate (APMA) in dimethylsulfoxide. The solution is stored at -20 °C.

Peptide hydrolysis buffer: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl₂, 0.025 % Brij 35. The solution is stable for several weeks at 4 °C.

Stock solution of peptide substrate: 100 μM solution of Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg in 20 % dimethylsulfoxide. The solution is stored at -20 °C.

Stock solution of unquenched peptide: 10 μM solution of (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-NH₂ (Mca-Pro-Leu) in 20 % dimethylsulfoxide. The solution is stored at -20 °C.

Activation An aliquot of 19.5 μl procollagenase-3 is mixed with 0.5 μl APMA solution and the mixture is incubated for 90 minutes at 37 °C.

Assay protocol The activity of collagenase-3 is measured fluorimetrically with a synthetic internally quenched fluorescent substrate according to Knight *et al.* [10]. An excitation wavelength of 328 nm and an emission wavelength of 393 nm are set in an appropriate fluorimeter. The instrument is calibrated with the unquenched peptide Mca-Pro-Leu at a concentration corresponding to between 2 and 10 % hydrolysis of the protease substrate. Kinetic reactions are conveniently carried out in a constant volume of 2.5 ml. The substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg is diluted in peptide hydrolysis buffer to a concentration of 0.8 μM and equilibrated at a temperature of 37 °C. Aliquots of 1 μl to 2 μl of the activation mixture are then added and the increase in fluorescence is recorded over a time interval between 2 and 12 min. Activity units per ml enzyme solution are calculated according to the following equation:

$$\text{Activity U/ml} = \frac{c_{\text{Mca-Pro-Leu}} \cdot \Delta F_{\text{Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg}}}{F_{\text{Mca-Pro-Leu}} \cdot v_{\text{enzyme}} \cdot V_{\text{total}}}$$

$c_{\text{Mca-Pro-Leu}}$: Concentration of Mca-Pro-Leu used for calibration of the fluorimeter (μmoles/ml)

$F_{\text{Mca-Pro-Leu}}$: Fluorescence of Mca-Pro-Leu at the concentration $c_{\text{Mca-Pro-Leu}}$ used for fluorimeter calibration

$\Delta F_{\text{Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg}}$: Change in fluorescence during peptide hydrolysis per min

V : Volume of peptide hydrolysis reaction (2.5 ml)

v : Volume of added enzyme (0.001 ml to 0.002 ml)

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