Biodiagnostika
Oligonukleotide
Markierte Biomoleküle
BIOTEZ

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Note that this data sheet is not lot-specific. Please consult the vial label and the certificate of analysis for information on specific lots.

Matrix Metalloproteinase 9 (MMP 9) / TIMP1 Complex

(Progelatinase B Monomer / TIMP1 Complex)

Catalogue Number: 30 100 532 Package Size: 10 µg

1. Enzyme characteristics

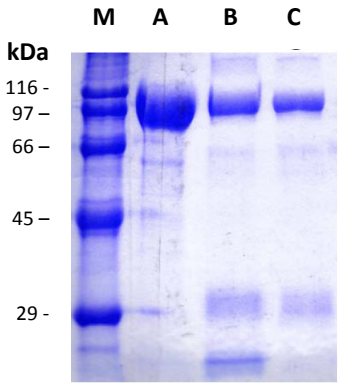
1.1 Molecular form: Progelatinase B complex is isolated from human blood. The protein is solubilized in 50 mM Tris-HCl, pH 7.0, 200 mM NaCl, 5 mM $CaCl_2$, 1 µM $ZnCl_2$, 0.05 % NaN_3, 0.05 % Brij-35.

1.2 Purity: >95% homogenous by electrophoresis and Western Blotting analysis.

1.3 Specific activity: The specific activity of MMP9/TIMP complex after trypsin activation is >300 mU/mg. 1 U is the activity that hydrolyzes 1 µmol peptide (7-methoxycoumarin-4-yl) acetyl-Pro-Leu-Gly-Leu-(3-[2, 4-dinitrophenyl]-L- 2, 3-diamino-propionyl)-Ala-Arg-NH_2 *(Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg)* within 1 min under the assay conditions as described.

1.4 Inhibitors: Gelatinase B is inhibited by tissue inhibitors of matrix metalloproteinases (TIMP) and by chelators of divalent cations as EDTA or o-phenanthroline.

1.5 Stability and storage: The enzyme is stable until the expiry date given on the label if stored at -70 °C. Repeated freezing and thawing should be avoided.



SDS- PAGE (reducing condition):
M: Protein Marker
A: MMP9
B: MMP9/ Lipocalin/ TIMP1
C: MMP9/ TIMP1

2. Applications

Purified progelatinase B monomer can serve as antigen standard in immunochemical analyses. Active gelatinase B is used to study the degradation of extracellular matrix proteins, to screen inhibitors of matrix metalloproteinases and to characterize inhibitor actions.

3. Introduction to structure and function of gelatinase B

Human progelatinase B consists of 668 amino acids with a calculated M_r of 76 240 Da. Due to N- and O-linked glycosylation the M_r in SDS-PAGE is about 92 kDa [2]. Within the protein sequence the following structural domains can be distinguished [2, 3]: An N-terminal propeptide, which confers latency to the proenzyme, a Ca^{2+}- and Zn^{2+}-ions binding catalytic domain containing an insertion of three repeats homologous to type II repeats in the gelatin-binding region of fibronectin, and a C-terminal hemopexin-like domain. Catalytic and hemopexin domains are connected by a proline-rich sequence with homology to sequences in collagens. The gelatin-binding region and the hemopexin domain confer specific macromolecular substrate binding to gelatinase B. The hemopexin domain of the latent enzyme binds TIMP-1 [2, 4, 5].

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Gelatinase B from neutrophil granulocytes displays three bands on SDS-PAGE (reducing condition) at 92, 29 and 25 kDa. The 92 kDa form represents the monomer, the 29 kDa protein band represents TIMP 1 and the 25 kDa protein which belongs to the lipocalin family and displays homology with the α2- macroglobulin related protein from rats [5]. These proteins are capable of forming complexes, including the binary MMP9/ TIMP1 complex, which behaves like the ternary MMP9/ Lipocalin/ TIMP1 complex that it is an inhibitor for active MMPs and, after activation, a gelatinase with a reduced activity.

Activation of progelatinase B can be mediated by proteases like stromelysin, cathepsin G, kallikrein and trypsin or by incubation with APMA [6]. In the presence of APMA the propeptide is not removed completely, however, and there occurs considerable C-terminal self-processing [6].
Active gelatinase B hydrolyses type IV and V collagens, gelatins, elastin, laminin and proteoglycan [6]. The enzyme is inhibited by inhibitors of matrix metalloproteinases (TIMP-1 and TIMP-2) [6]. Gelatinase B is secreted from macrophages, polymorphonuclear leukocytes, keratinocytes and many tumor cells [7, 8, 9]. It is detected in human plasma [10] and saliva [11]. Gelatinase B is involved in physiological processes as angiogenesis, wound healing, bone remodeling, migration of macrophages and leukocytes [12]. Hydrolysis of type IV collagen and other matrix proteins in basement membranes by gelatinase B contributes to tumor cell invasion [13] and aortic aneurysm formation [14].

4. Trypsin activation of gelatinase B and measurement of catalytic activity

4.1 Preparation and stability of solutions

Activation buffer: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$.

Trypsin solution: 0.50 mg TPCK-trypsin / ml activation buffer. The solution is stored in aliquots at -20°C.

Aprotinin solution: 1 mg aprotinin / ml activation buffer. The solution is stored at - 20°C.

Peptide hydrolysis buffer: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 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-NH_2 (Mca-Pro-Leu) in 20 % dimethylsulfoxide. The solution is stored at -20°C.

4.2 Activation: Progelatinase (and the complexes) needs activation which can be performed by following methods. The activation methods will result in the same catalytic efficiency against a peptide or gelatin substrate:

A) Incubation with 100 µg/ml TPCK-trypsin for 30 min, at 37°C. If required the trypsin can be inhibited with 1 µl of 1 mg/ml aprotinin solution and incubated for 10 min at 25°C.
B) Incubation with 2 mM (final concentration) aminophenylmercuric acetate (APMA) for 1-2 hours at 37°C in ACA buffer (50 mM Tris/HCL, pH 7.0; 200 mM NaCl; 1 µM $ZnCl_2$; 5 mM $CaCl_2$; 0.05% NaN_3.
C) If one is working with ProgelatinaseB/TIMP 1 complex it is recommend that Stromelysin- 1 (MMP 3) is used to activate the Progelatinase B. Incubation at 37°C for 2 hours at a 40:1 ratio of Progelatinase B to Stromelysin-1 will remove only the propeptide to give the active 84 kDa form of the enzyme.

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4.3 Assay protocol: The activity of gelatinase B is measured fluorimetrically with a synthetic internally quenched fluorescent substrate according to Knight *et al.* [15].
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 5 µl to 10 µl of the activation mixture are than 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:

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

$c_{\text{Mca-Pro-Leu}}$: 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 (0.005 ml to 0.010 ml)

5. References

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