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

Membrane-type 3 Matrix Metalloproteinase (MT3-MMP, MMP 16) Reombinant Catalyic Domain
Catalogue Number: 30 100 302 Package Size: 10 µg / 50 µl
Catalogue Number: 30 100 303 Package Size: 200 µg / 1 ml

1. Enzyme characteristics

1.1 Molecular form: The catalytic domain of MT3-MMP is produced by activation of a recombinant soluble proform of MT3-MMP purified from *E. coli*. The polypeptide sequence extends from Leu<sub>91</sub> to Arg<sub>279</sub> of mature human MT3-MMP and comprises the following 189 amino acids:

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The calculated M<sub>r</sub> of recombinant catalytic domain is 21 kDa.

1.2 Purity: MT3-MMP appears as a major band at 23 kDa in SDS-PAGE and represents about 80% of the protein in the preparation. The enzyme is solubilized in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM CaCl<sub>2</sub>, 1% glycerin, 0.1% Triton X-100.

1.3 Specific activity: The specific activity of MT3-MMP catalytic domain is ≥ 75 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<sub>2</sub> (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg) within 1 min under the assay conditions described below.

1.4 Inhibitors: The catalytic domain of MT3-MMP is inhibited by tissue inhibitor of matrix metalloproteinases-2 (TIMP-2) and by chelators of divalent cations like EDTA or o-phenanthroline.

1.5 Stability and storage: MT3-MMP is stable until the expiry date given on the label if stored at -70 °C. The enzyme can be kept at -20°C for several weeks and in ice for 2 days without significant loss of activity. The catalytic domain of MT3-MMP can show a weak autoproteolytic activity at ≥30°C and at concentrations ≥ 0.2 µg/µl. For incubations longer than 1 h it is recommended to dilute the enzyme to ≤ 0.1 µg/µl. Repeated freezing and thawing should be avoided.

2. Applications

The recombinant catalytic domain of MT3-MMP is used to study the activation of progelatinase A (matrix metalloproteinase inhibitors and characterization of inhibitor action).
3. Introduction to structure and function of membrane-type 3 matrix metalloproteinase

Matrix metalloproteinases (MMPs) are Zn\(^{2+}\) - and Ca\(^{2+}\)-dependent endopeptidases which function in the turnover of extracellular matrix components [1]. Presently fifteen secreted MMPs and six membrane-type MMPs are known to be expressed in vertebrates. Human MT3-MMP consists of 607 amino acid residues with a calculated M₉ of 69,528 Da [2]. The following domains and sequence regions are distinguished in MT3-MMP: Prodomain (Ala\(^1\) - Arg\(^88\)), catalytic domain (Tyr\(^89\) - Gly\(^260\)), junction between catalytic domain and hemopexin domain (Pro\(^261\) - Lys\(^308\)), hemopexin-like domain (Pro\(^309\) - Cys\(^501\)) and C-terminal sequence (Asp\(^502\) - Val\(^607\)) with transmembrane segment.

MT3-MMP is expressed in adult human lung, brain, heart, placenta, ovary, liver and spleen [2,3]. The enzyme activates progelatinase A (72 kDa type IV procollagenase) [2,3]. A recombinant deletion variant consisting of MT3-MMP catalytic domain and part of the hemopexin domain hydrolyzes fibronectin and collagen III [4]. The activity of the deletion variant is poorly inhibited by tissue inhibitor of matrix metalloproteinases-1 (Timp-1) but efficiently inhibited by Timp-2 [4].

4. Measurement of MT3-MMP activity

4.1 Preparation and stability of solutions

**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 % dimethylsulfoxid. 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 Assay protocol

The activity of MT3-MMP catalytic domain is measured fluorimetrically with a synthetic internally quenched fluorescent substrate according to Knight et al. [5]. 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 2 µl to 4 µl of matrix metalloproteinase are then added and the increase in fluorescence is recorded over a time interval between 2 and 12 min. Activity units are calculated according to the following equation:

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\text{Activity U/ml} = \frac{\Delta F_{\text{Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg}} \times c_{\text{Mca-Pro-Leu}} \times V}{1000 \times F_{\text{Mca-Pro-Leu}} \times v}
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- \(c_{\text{Mca-Pro-Leu}}\): Concentration of Mca-Pro-Leu used for calibration of the fluorimeter (M)
- \(F_{\text{Mca-Pro-Leu}}\): 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 (0.002 ml to 0.004 ml)

5. References