MMP-1 proenzyme (human fibroblasts)

MMP-1, the classical matrix metalloproteinase, is expressed by a large number of cell types. As an example, in basal keratinocytes migrating across the dermal matrix the enzyme is invariably expressed and cleaves fibrilliar collagen type I. It was shown that the interaction of the $\alpha_2\beta_1$ integrin with dermal collagen mediates induction of MMP-1 in keratinocytes at the onset of healing and that the activity of MMP-1 is needed to initiate cell movement. The cleavage of dermal collagen provides keratinocytes with a mechanism to maintain their directionality during reepithelialization (Pilcher et al. 1997).
The MMP-1 zymogens of ~56/52kDa can be activated by a stepwise mechanism through which sequential processing events occur in the propeptide region. Both proenzymes can be activated by limited digestion with trypsin or by treatment with APMA generating their respective active enzyme forms of ~46/42kDa (Wilhelm et al. 1986).

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Ordering Information

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ALX-200-418-C005	5µg

Manuals, SDS & CofA

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Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended.
Handling	Avoid freeze/thaw cycles.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Matrix metalloproteinase 1, Interstitial collagenase, Fibroblast collagenase
Concentration	~80µg/ml (Pierce-BCA)
Formulation	Liquid. In 50mM TRIS-HCl, pH 7.0, 300mM NaCl, 5mM $CaCl_2$, 1µM $ZnCl_2$, 0.05% BRIJ35 and 0.05% sodium azide.
MW	~56kDa.
Purity	≥90% (SDS-PAGE, Western blot)
Purity Detail	No other MMP contaminants are detectable.
Source	Isolated from human rheumatoid synovial fibroblasts. Requires activation.
Specific Activity	≥100mU/mg protein (Y. Masui, et al.; Biochem. Med. **17**, 215 (1977)). One unit is defined as the amount of enzyme that hydrolyzes 1µmol Dnp-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg-OH per min. at 37°C, pH 7.0. APMA activation 1 hour at 37°C; enzyme dilution for assay 1:10/5-10µl for enzyme activity assay – incubation time 30min.

Technical Info / Product Notes	**Activity:** We recommend to dilute this enzyme preparation at least 1:10 and to take 5µl or less for the determination of activity. Specific activity can be assayed with the synthetic substrate N-(2,4)-dinitrophenyl-Pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg (Dnp-peptide) (Masui et al.). Substrate concentration should be 0.5mg/ml in buffer 50mM TRIS-HCl, pH 7.0, 200mM NaCl, 5mM $CaCl_2$, 1µM $ZnCl_2$, 0.05% BRIJ35, 0.05% sodium azide, containing 0.05mg/ml albumin. One unit MMP catalyzes the hydrolysis of 1µmol Dnp-peptide/min at 37°C and pH 7.0. We recommend to employ the fluorogenic substrate (7-Methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Leu-N-β-Dnp-L-α,β-diaminopropionyl-Ala-Arg-NH_2) (Knight et al. 1992). The hydrolysis of the Gly-Leu bond separates the highly fluorescent (7-Methoxycoumarin-4-yl)acetyl group from the 2,4-dinitrophenyl resulting in an increase of fluorogenic intensity. The substrate should be kept as a 9.15mM stock solution in DMSO (10mg/ml). In the assay the substrate concentration should be ~25mM. The assay can be performed in a 96-well microtiter plate (200µl per well) suitable for fluorogenic measurements (excitation wavelength of 328nm; emission wavelength of 393nm). **Activation:** Do not dilute enzyme for activation! Activation is required by trypsin (2µl trypsin; 1mg/ml) for 10-20 min at 37°C and stopped by the addition of 10µl trypsin-inhibitor (2mg/ml) or aprotinin or TLCK. Activation can also be done by 2mM (final concentration) APMA for 60 min. at 37°C. **Inhibitors:** Only the activated and not the latent forms of wild-type MMP-1 protein is able to form a complex with TIMP-1. Quite in contrast to MMP-2 (gelatinase A) and MMP-9 (gelatinase B), in MMP-1 the C-terminal hemopexin domain does not interact with TIMP-1. The integrity of the catalytic domain of MMP-1 and its ability to bind Zn^{2+} is absolutely required for complex formation with TIMP-1, which further underlines the importance of this region for proper regulation of enzymatic activity of MMP-1 (Vallon et al. 1997). Therefore, the enzyme is also inhibited by chelators of divalent cations like EDTA or o-phenantroline.
UniProt ID	P03956

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