

Recombinant Human MMP-12 (carrier-free)

Catalog# / Size	764202 / 10 µg 764204 / 25 µg
Regulatory Status	RUO
Other Names	Macrophage metalloelastase (MME), Matrix metalloproteinase 12
Description	<p>Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade components of the extracellular matrix (ECM) and play essential roles in various physiological and pathological processes such as morphogenesis, differentiation, angiogenesis, tissue remodeling, and tumor invasion. A typical MMP consists of a propeptide of about 80 amino acids, a catalytic metalloproteinase domain of about 170 amino acids, a linker peptide of variable lengths and a hemopexin domain of about 200 amino acids. The zinc binding motif HEXH-HXXGXXH in the catalytic domain, and the "cysteine switch" motif PRCGXPD in the propeptide are common structural signatures. MMP-12 (macrophage elastase) is a 54 kDa proenzyme consisted of propeptide, catalytic metalloproteinase domain, and hemopexin domain. The predicted human 54-kD protein is processed by loss of both N- and C-terminal residues to a 22-kD mature form. It is a major proteinase that degrades elastin. MMP-12 can also degrade a broad spectrum of substrates, including type IV collagen, fibronectin, laminin, vitronectin, proteoglycans, chondroitin sulfate, myelin basic protein, alpha 1-antitrypsin, and plasminogen. MMP-12 is mainly produced by macrophages and has been shown to be associated with inflammatory skin diseases, atherosclerosis, aneurysms, and cancers. MMP12 can exhibit both proinflammatory and anti-inflammatory activity in a tissue- or disease context-dependent manner. The purified recombinant MMP-12 is consisted of pro-peptide and catalytic domain.</p>

Product Details

Source	Human MMP12, amino acids Leu17-Gly263 (Pro-peptide and catalytic domain; Accession #NM_006287) with a C-terminal TG-8H-GGQ tag was expressed in CHO cells.
Molecular Mass	The 260 amino acid recombinant protein has a predicted molecular mass of approximately 29.2 kDa. The non-reduced and DTT-reduced proteins migrate at 32 - 35 kDa by SDS-PAGE.
Purity	> 90%, as determined by Coomassie stained SDS-PAGE
Formulation	0.22 µm filtered protein solution is in 10 mM MES, 5 mM CaCl ₂ , 150 mM NaCl and 50% glycerol at pH 5.5.
Endotoxin Level	Less than 1.0 EU per µg of protein as determined by the LAL method
Concentration	10 and 25 µg sizes are bottled at 200 µg/mL. 100 µg size and larger sizes are lot-specific and bottled at the concentration indicated on the vial. To obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.
Storage & Handling	Unopened vial can be stored at -20 °C or -70°C for six months. For maximum results, quick spin vial prior to opening. Avoid repeated freeze/thaw cycles.
Activity	Human MMP-7 cleaves a fluorogenic peptide substrate Mca-PLGL(Dpa)AR with a specific activity value > 750 pmol/µg/min
Application	Bioassay
Application Notes	Human MMP-12 Activity assay

Human MMP-12 (hMMP-12) activity is measured by its ability to cleave a fluorogenic peptide substrate Mca-PLGL(Dpa)AR after its activation in the presence of p-Aminophenylmercuric acid. The increase of the product is monitored by increase in intensity of fluorescence at 405 nm with excitation at 320 nm. This protein is in the latent form and needs to be activated for bioassay.

Materials

1. Assay Buffer: TCNB (50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% Brij-35, pH 7.5)
2. Recombinant Human MMP-12
3. Mca-PLGL(Dpa)AR (AnaSpec Cat # AS27076; 2 mM in DMSO)

4. P-Aminophenylmercuric acid (Calbiochem Cat# 164610; 100 mM in DMSO)

Activity assay procedures

1. Dilute hMMP-12 in the assay buffer at 100 µg/mL.
2. Activate hMMP-12 by adding APMA to a final concentration of 1 mM.
3. Incubate the activating mixture for 1 hr at 37 °C.
4. Dilute the activated hMMP-12 to 0.4 µg/mL (0.4 ng/µL) in Assay Buffer.
5. Dilute the substrate at 20 µM in Assay Buffer.
6. Load into a well plate 50 µL of the 0.4 ng/µL hMMP-12 and start the reaction by adding 50 µL of 20 µM Substrate. Include a substrate blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate without any hMMP-12.
7. Read the product formation by measuring 320/ 405 nm (Excitation/Emission) in kinetic mode for 5 minutes.

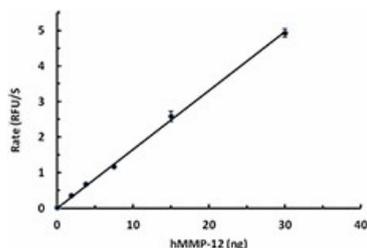
The final hMMP-12 concentration is 0.2 µg/mL (0.2 ng/µl, 20 ng)
The final concentration of the substrate is 10 µM.

BioLegend carrier-free recombinant proteins provided in liquid format are shipped on blue-ice. Our comparison testing data indicates that when handled and stored as recommended, the liquid format has equal or better stability and shelf-life compared to commercially available lyophilized proteins after reconstitution. Our liquid proteins are verified in-house to maintain activity after shipping on blue ice and are backed by our [100% satisfaction guarantee](#). If you have any concerns, contact us at tech@biolegend.com.

Antigen Details

Structure	Monomer
Distribution	High expression in macrophage; Moderate expression in Fibroblast cell
Function	Wound healing; Extracellular matrix disassembly
Interaction	Extracellular matrix
Ligand/Receptor	TIMP-1, TIMP-2, TIMP-4
Bioactivity	Regulate blood coagulation by inhibit FX _a and FVII _a
Biology Area	Angiogenesis, Cell Adhesion, Cell Biology, Neuroinflammation, Neuroscience, Stem Cells
Molecular Family	Enzymes and Regulators
Antigen References	<ol style="list-style-type: none">1. Liu S-L <i>et al.</i> 2015. <i>Sci. Rep.</i> 5:17189.2. Laurenzana A <i>et al.</i> 2014. <i>Oncotarget.</i> 5:3711.3. Bellac CL <i>et al.</i> 2014. <i>Cell. Rep.</i> 9:618.4. Lee JT <i>et al.</i> 2014. <i>Endocrinology.</i> 155:3409.5. Tallant C <i>et al.</i> 2010. <i>Biochem. Biophys. Acta.</i> 1803:20.6. Nagase H, <i>et al.</i> 2006. <i>Cardiovasc. Res.</i> 69(3):562-73.7. Rundhaug JE, 2005. <i>J. Cell. Mol. Med.</i> 9:267
Gene ID	4321

Product Data



hMMP-12 (50 µg/mL) was activated in the presence of 1 mM APMA for 1 hr at 37 °C. The activity of hMMP-12 was measured with 10 µM of fluorogenic MMP substrate, Mca-PLGL-Dpa-AR-NH₂, in the presence of 1.88, 3.75, 7.5, 15, 30 ng of activated hMMP-12. The activity of activated hMMP-12 is greater than 750 pmole/min/µg.

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BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587