Human HME-MMP12 ELISA kit
(DDXK-E-MMP12-2)

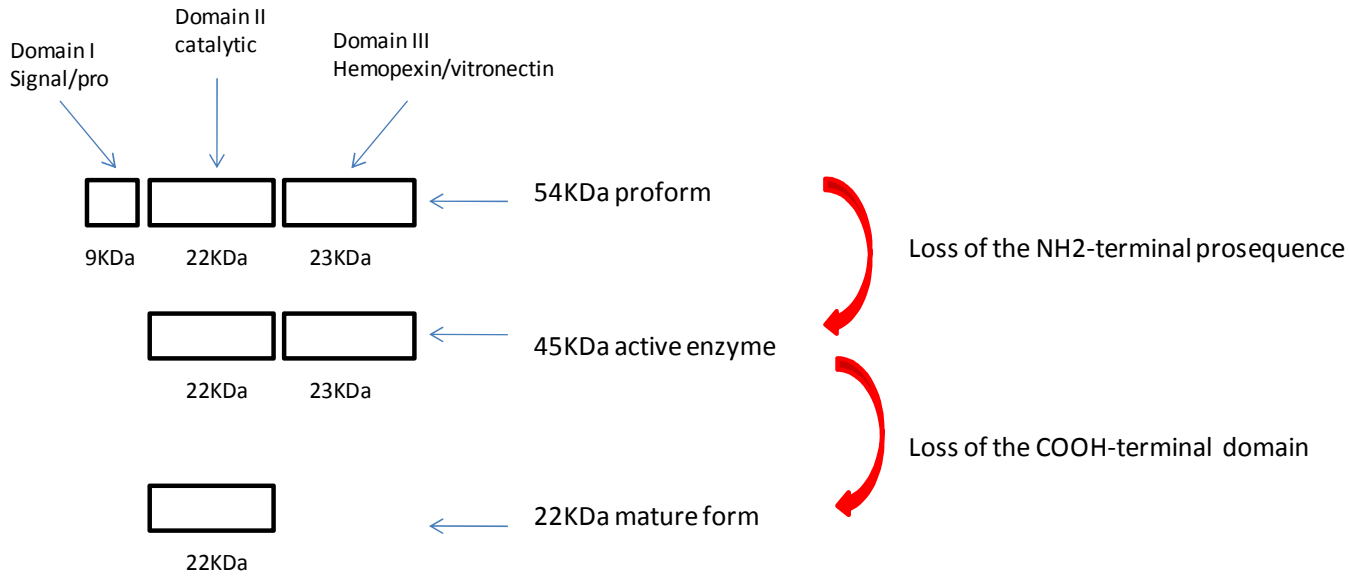
Background

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes. HME-MMP-12, also called metalloelastase, is critical for invasion and destruction in pathologies such as aneurysm and emphysema. HME mRNA and protein were previously described as expressed in human alveolar macrophages *(Curci JA et al, 1998, J Clin Invest, 102(11):1900-10)*. The predicted molecular mass of the HME proenzyme is 54 kDa and readily undergoes NH2- and COOH-terminal processing to an active 45KDa form and a mature 22 kDa form. HME is a unique human metalloproteinase that displays elastolytic activity. We have isolated HME-MMP12 cDNA from a human $CD34^+$-derived DC (GM-CSF+TNFα) library. Expression analysis revealed HME-MMP12 expression in lung and placenta. Anti-HME-MMP12 monoclonal antibodies were obtained after mice immunization with HME-MMP12-transfected eukaryotic cells. These antibodies were used to set up a quantitative immunoassay for the detection of human HME-*MMP12 (Demedts IK et al, 2006; Thorax, 61:196-201; Lapan et al, BMC Pulmonary Medicine, 2010, 10:40).*

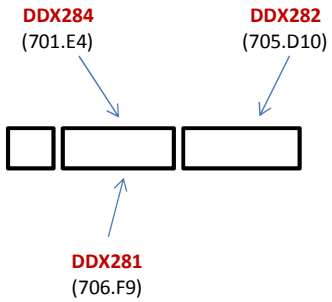
Intended use

The human HME-MMP12 ELISA kit is for quantitative determination of HME-MMP12 concentrations in human cell culture supernatants and biological fluids (plasma, serum, sputum). This kit is for research purpose only.

HME-MMP12 domain structures and maturation steps were described by ***Shapiro et al, JBC, 1993, 268, 23824-23829,*** and are represented below.



Dendritics has developed a series of monoclonal antibodies raised against HME-MMP12. Several rounds of experiments were performed to identify the recognition site for each antibody. These analyses resulted in the following scheme:

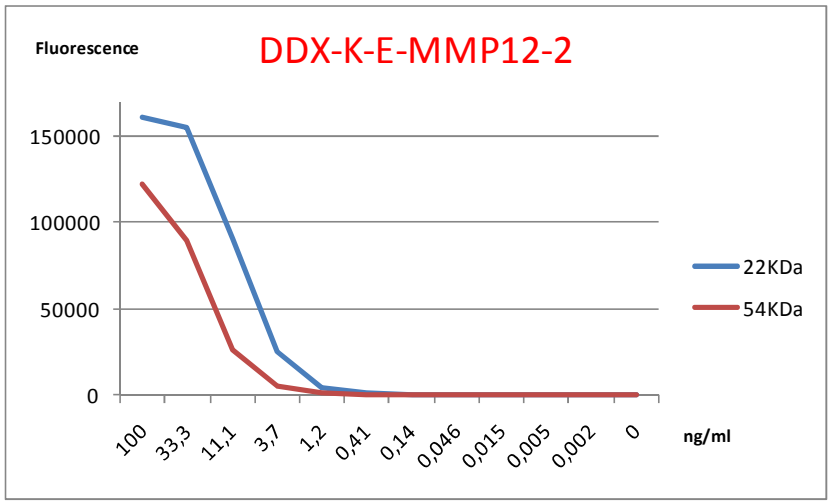


The Elisa kit using the DDX284-DDX281 combination (**DDXK-E-MMP12-2**) will reveal:

the proenzyme form (**54KDa**),
the active form (**45KDa**)
and the mature form (**22KDa**).

Quantification of rhMMP12 from R&D systems

A recombinant form of the human MMP12 (R&D systems, 917-MP) was either used untreated or activated for 30 hours at 37°C. 2 bands corresponding to the proform (54KDa) and the mature form (22KDa) were visualized by Blue Comassie staining. Each form was quantified using the DDXK-E-MMP12-2 kit:



Kit contents

Capture Antibody: 0.5mg/mL of mouse anti-HME-MMP12 monoclonal antibody (clone 701E4.03).

Detection Antibody: 0.5mg/mL of HRPO-conjugated anti-HME-MMP12 mouse monoclonal antibody (clone 706F9.01).

Standard: Each vial contains a solution of recombinant HME-MMP12 (***concentration indicated on the vial***) produced, purified and concentrated from transfected eukaryotic cells. A 8 point standard curve using 2-fold serial dilutions in sample dilution buffer, starting at a concentration of 1µg/ml is recommended.

Storage

All the reagents should be aliquoted before storage. Minimize repeated freeze and thaw. Refer to expiration date on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot number.
Capture and detection antibodies storage: -20°C; Standard storage: -80°C

Materials and reagents required but not provided

96well-plate Nunc Maxi Sorp
50mM Carbonate buffer pH9.6
PBS-1% BSA-0.05%Tween20
TMB super sensitive HRP (TMBS100-0500, TEBU-BIO)
Multichannel pipettes and pipette tips
A standard microplate reader (620nm)

Sensitivity

The minimum detectable dose of human HME-MMP12 was determined to be <50pg (Demets et al, 2006).

Principle of the assay

The human HME-MMP12 ELISA kit is for the quantitative determination of human HME-MMP12 in human cell culture supernatants, plasma, serum, and various biological fluids (sputum).
This ELISA kit contains the specific components required for the development of human HME-MMP12 sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on 3 X 96-well plates.
The DDX HME-MMP12 ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). The capture monoclonal antibody specific for HME-MMP12 is coated on a 96-well plate. Standards and samples are added to the wells, and any HME-MMP12 present binds to the immobilized antibody. The wells are washed and a HRPO-conjugated anti-HME-MMP12 monoclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. To produce color in proportion to the amount of HME-MMP12 present in the sample, TMB substrate solution is loaded and absorbance is measured at 620 nm.

MMP12 total protein Elisa protocol

The protocol described the main steps of the procedure. Before setting up the Elisa assay, reading the following references is strongly recommended:

Demets IK et al, Thorax 2006; 61 :196-201

Lapan et al, BMC Pulmonary Medicine, 2010, 10:40

For the recognition of the 54 KDa form in sputum samples, a pre-treatment with DTT is required (Demets IK et al, Thorax 2006; 61: 196-201)

Pre-warm all the reagents to room temperature prior to setting up the assay

1. Coat the capture antibody, Dendritics cat#DDX0284 at 2.5µg/ml, 120µl/well in Elisa coating buffer (50mM carbonate/bicarbonate buffer pH9.6). Seal the plate and incubate overnight at room temperature
2. Wash with 200µl PBS-0.05% Tween20 at RT
3. Add 100µl/well of samples or standards diluted in PBS-1% BSA-0.05% Tween20
4. Incubate 2h at 37°C
5. Wash with 200µl PBS-0.05% Tween20 at RT
6. Add 100µl/well of DDX0281-HRPO antibody diluted at 3µg/ml in PBS-1% BSA-0.05% Tween20
7. Incubate 1h at 37°c
8. Wash 3 times with 200µl/well in PBS-0.05% Tween20
9. Add 100µl/well TMB Super Sensitive HRP microwell substrate (Tebu Bio Laboratories)
10. Determine the optical density of each well using a microplate reader set at 620nm.

Standard curve and specificity

Specificity testing showed that there was no detection of other related MMP (1-3-9) using DDX anti-MMP12 monoclonal antibodies

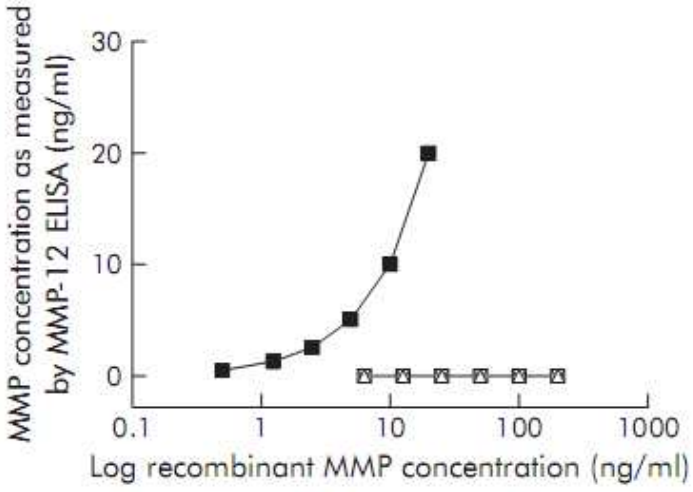


Figure 2 Specificity of the ELISA for MMP-12. Detection of a serial dilution of recombinant MMP-12 protein by the ELISA (■). No recombinant MMP-1 (△), MMP-3 (▽), or MMP-9 (□) were detected with the ELISA, even at high concentrations.

(Demets et al, 2006)

Troubleshootings

To obtain good and reproducible results, usage of sterile reagents and clean materials is strongly recommended. All basic reagents such as washing and dilution buffers, water, must be devoid of contamination.

To ensure pH stability, incubation at 37°C should be performed in a humidified atmosphere of 5% CO_2

Problems	Possible Sources	Solutions
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Inappropriate storage	Aliquot standard and store at -70°C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature or timing	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples