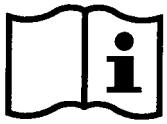
Product information



Userś Manual

MMP-3 ELISA

Enzyme Immunoassay for the determination of MMP-3 concentration in human serum.

REF AE29004

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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2015-09-23 DWZ
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1 INTENDED USE

This MMP-3 ELISA is a solid phase enzyme immunoassay with two different monoclonal antibodies against human MMP-3 for the precise measurement of MMP-3 concentration in human serum.
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2 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease which is primarily characterised by joint inflammation and leads to a loss of joint function and severe disability if the progress of the disease is not tackled in time.
Matrix metalloproteinase-3 (MMP-3, stromelysin-1) belongs to the family of metalloproteinases. It is predominantly expressed by connective tissue cells and plays an important role in the material conversion processes of the extracellular matrix (ECM). MMP-3 can break down many components of the ECM such as proteoglycans, fibronectin, laminin and various other collagens. As a result of this large range of substrates and the ability to activate other degrading enzymes, including other matrix metalloproteinases, MMP-3 plays a key role in the physiological and pathological processes of tissue remodelling.
MMP-3 is secreted in an inactive form (pro-MMP-3, 52 kDa) and activated by limited proteolysis by endopeptidases. Active MMP-3 (45 kDa and 35 kDa) can be inhibited as a result of binding to tissue inhibitors of matrix metalloproteinases (TIMP) or α2-macroglobulin. Regulating MMP-3 activity is necessary to prevent the destruction of the ECM and to preserve the physiological equilibrium in tissue remodelling processes. Accordingly, individuals with various pathological conditions such as cancer, atherosclerosis and arthritis have elevated MMP-3 concentrations.

3 PRINCIPLE OF THE TEST

Principle of the test

The **1:10** diluted serum samples are incubated in cavities which are coated with a monoclonal anti-human MMP-3 antibody. In this process, MMP-3 from the sample serum binds to the antibody on the plate; unbound serum components are washed away in the subsequent washing step. A monoclonal anti-human MMP-3 antibody, which is marked with horseradish peroxidase (conjugate), is then added. During incubation, this binds to the previously formed antibody-MMP3-complex. Unbound conjugate is removed in the subsequent washing step. Bonded MMP-3 is detected by an enzymatic color reaction (blue) of the substrate, which is stopped using diluted acid (sudden color change to yellow). The intensity of the color development of the chromogen depends on the amount of conjugate bound to the antibody-MMP-3-complex and is therefore directly proportional to the MMP-3 concentration in the serum.

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4 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of calibrators: 0, 5, 20, 50, 100, 200 ng/ml. Purified human MMP-3, BSA, sodium azide < 0.1% (preservative)
Conjugate	1 x 15ml	Red	Red	Anti-human MMP-3 marked with horseradish peroxidase, BSA
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

5 STORAGE CONDITIONS

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

6 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only, not for use in diagnostic procedures.

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7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 SAMPLE COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test. Plasma samples should not be used in this test.
2. Samples should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Samples which cannot be assayed within 24 hours of collection should be frozen at –20 °C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Samples which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

9 PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

10 PREPARATION OF REAGENTS AND SAMPLES

All reagents should be brought to room temperature (18 °C - 25 °C) before use.

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:10 with sample buffer (1x)

e.g. 450 µl sample buffer (1x) + 50 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

carefully remove liquid by tapping the plate on filter paper. Pipette 300 µl diluted wash buffer in each cavity, wait 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

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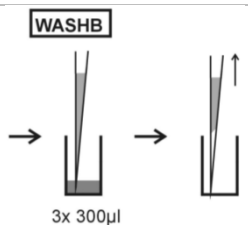
11 ASSAY PROCEDURE

We suggest pipetting calibrators, controls and samples as follows:

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

Cal A: calibrator A
Cal B: calibrator B
Cal C: calibrator C
Cal D: calibrator D
Cal E: calibrator E
Cal F: calibrator F
PC: positive control
NC: negative control
P1: sample 1
P2: sample 2
P3: sample 3

Step	*Description*
1.	Ensure preparations from step 10 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with the intended quantitative interpretation of the results:
	Calibrators, controls, and samples
3.	 Pipette in each case 100 µl in the provided cavities as described above: • Calibrators (Cal A to Cal F) • Negative control (NC) and positive control (PC) and • Diluted samples (P1, P2, ...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x in each case with 300 µl washing buffer (diluted 1:50).

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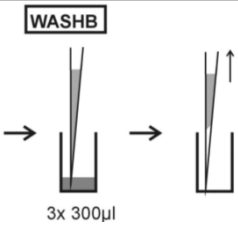
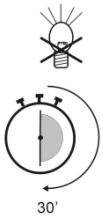
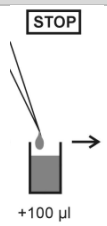
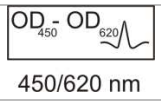
	CONJUGATE	
6.		Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.		Wash 3x in each case with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

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12 INTERPRETATION

The **quantitative evaluation** is based on a standard curve in which the optical density of the calibrators (y-axis) is plotted against the concentration in ng/ml (x-axis). A log-linear plot and a 4-parameter fit is recommended for the evaluation. Using the curve, the MMP-3 concentration in ng/ml is established from the optical density of the sample.

MMP-3	Normal range	Borderline	Positive
Women	0 – 20 ng/ml	20 – 30 ng/ml	> 30 ng/ml
Men	0 – 40 ng/ml	40 – 50 ng/ml	> 50 ng/ml

Evaluation example

This example must not be used to interpret sample results!

Calibrators	OD 450/620 nm	CV % (variance)
0 ng/ml	0.033	2.2
5 ng/ml	0.105	4.0
20 ng/ml	0.336	1.7
50 ng/ml	0.727	1.2
100 ng/ml	1.328	1.6
200 ng/ml	2.228	3.7

Example calculation

Sample	Replication (OD)	Mean (OD)	Result (ng/ml)
P 01	0.264/0.258	0.261	17.2
P 02	1.323/1.326	1.325	97.5

Samples that are above the highest calibrator value should be reported as > max. They should be diluted accordingly and be re-evaluated, taking the dilution factor into account. Samples lower than the measurement range should be reported as < min.

For batch-specific data please see the attached QC certificate. Medical laboratories should perform in-house quality controls with their own controls and/or pooled sera according to national legislation.

It is recommended that each laboratory works out its own normal values, based on its own technology, controls, equipment and population.

If the control values do not meet the validation criteria, the test is invalid and must be repeated.

The following technical data should be reviewed: expiry dates of the reagents, storage conditions, pipettes, used equipment, photometer, incubation conditions and washing methods.

If the tested samples reveal unusual values or deviations, or if the validation criteria are not met for inexplicable reasons, please contact the IBL-America.

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13 TECHNICAL DATA

Sample material:	Serum
Sample volume:	100 µl of a 1:10 sample dilution with 1x sample buffer
Total incubation period:	90 minutes at 20-26°C/68-78.8°F.
Measurement range:	0-200 ng/ml
Analytical sensitivity:	5 ng/ml
Storage:	at 2-8°C/35-46°F in original bottles.
Number of determinations:	96 tests

14 PERFORMANCE DATA

14.1 Analytical Sensitivity

80 tests with the sample buffer in this MMP-3 ELISA gave a Limit of Blank of 4 ng/ml, and testing 8 sera at low MMP-3 concentration with 8 repetitions gave a Limit of Detection of 5 ng/ml.

14.2 Specificity

The microtiter plates are coated with murine monoclonal antibodies against human MMP-3.

Cross reactivities with other antigens could not be detected.

14.3 Linearity

For selected sera, a linear relationship between dilution and antibody concentration could be determined in this test. As a result of the heterogeneity of human serum, however, it is not excluded that individual sera display non-linear behavior.

Sample no.	Dilution	Measured concentration (ng/ml)	Expected concentration (ng/ml)	Recovery (%)
1	1 / 10	178.4	178.4	100.0
	1 / 20	86.1	89.2	96.5
	1 / 40	45.0	43.0	104.6
	1 / 80	23.2	22.5	103.0
2	1 / 10	88.8	88.8	100.0
	1 / 20	44.1	44.4	99.4
	1 / 40	22.8	22.1	103.1
	1 / 80	11.3	11.4	99.5

14.4 Precision

To control the assay precision, five sera in different regions of the standard curve were used to establish the variance (intra and interassay variance and the lot-to-lot variance), in which the reproducibility was investigated in 5 rounds, each with 8 repetitions. The lot-to-lot variance was investigated, whereby five sera were investigated from 3 different batches in 8 repetitions.

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Intraassay		
Sample no.	**Mean (ng/ml)**	**CV (%)**
1	12.6	4.9
2	30.5	3.6
3	59.3	3.2
4	101.0	3.6
5	195.1	3.4

Interassay		
Sample no.	**Mean (ng/ml)**	**CV (%)**
1	12.6	7.6
2	30.5	4.5
3	59.3	4.5
4	101.0	4.8
5	195.1	4.6

14.5 Calibration

In the absence of an international reference standard, the AESKULISA MMP-3 is calibrated against defined quantities of purified, recombinant human MMP-3 (using SEC-MALS established purity: > 99%). The results are given in ng/ml.

14.6 Recovery

Recovery was determined by adding various, defined quantities of recombinant human MMP-3 to different human sera. The following recovery rates were established by means of linear regression:

Serum sample	% recovery	Coefficient of determination R^2
1	101.4	0.9934
2	91.8	0.9938
3	88.5	0.9923

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Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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