EAGLE BIOSCIENCES

# MMP-7
# (Matrix Metalloproteinase 7)
# ELISA

Catalog Number: MM731-K01
1 x 96 Wells
*For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.*
*v. 4.2 (1.02.18)*

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## INTENDED USE

The Eagle Biosciences MMP-7 ELISA Kit is intended for the quantitative determination of the Cancer Antigen MMP-7 concentration in human serum. The Matrix Metallo-proteinase 7 (MMP-7) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

## SUMMARY AND EXPLANATION

MMP-7 proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. The MMP-7 enzyme degrades proteoglycans, fibronectin, elastin and casein and differs from most MMP family members in that it lacks a conserved C-terminal protein domain. The enzyme is involved in wound healing, and studies in mice suggest that it regulates the activity of defensins in intestinal mucosa. The gene is part of a cluster of MMP genes which localize to chromosome 11q22.3.

## PRINCIPLE OF THE TEST

The Eagle Biosciences MMP-7 ELISA test kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact MMP-7 molecule for solid phase immobilization (on the microtiter wells). Standards, calibrators, and samples are incubated with the biotin conjugated anti-MMP-7 monoclonal antibody and the solid phase antibody on the plate simultaneously. Wells are then washed and incubated with Streptavidin conjugated to HRP which is used as a reporting agent. Excess streptavidin-HRP is then washed off and a solution of TMB Reagent is added and incubated resulting in the development of a blue color if MMP-7 is present. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of MMP-7 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

## SPECIMEN REQUIREMENTS

Serum specimen collected in a no additive tube only and centrifuged after clotting. Run specimen within 8 hours of collection. If not analyzed with 8 hours specimen must be frozen at $\geq$-20°C until test is performed. Long-term specimen stability is yet to be determined. If frozen, samples must come to room temperature and mix thoroughly before analysis.

## MATERIALS PROVIDED

| | | |
|---|---|---|
| 1. | Microtiter plate coated with Monoclonal anti-MMP-7 | |
| 2. | MMP-7 reference standards: 6 vials (ready to use) | 0.35ml |
| 3. | Calibrators 1 and 2: 1 vial each (ready to use) | 0.35ml |
| 4. | Biotin-labeled monoclonal anti-MMP-7 antibody | 6ml |
| 5. | 10X Streptavidin-HRP (add 10ml $H_2O$ for 1X) | 1.1ml |
| 6. | TMB Reagent (One-Step) | 11ml |
| 7. | Stop Solution | 11ml |
| 8. | 10X Wash Concentrate | 30ml |

## MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. 1X PBS
3. Precision pipettes
4. Disposable pipette tips
5. Shaking incubator capable of holding temperatures of 30°C
6. ELISA reader capable of reading absorbance at 450nm
7. Absorbance paper or paper towel

## STORAGE AND STABILITY

1. Store the MMP-7 calibrators and standards are stable for up to 3 months at 2-8°C.
2. Keep microplate sealed in a dry bag with desiccants, store at 2-8°C.
3. Do not expose reagents to heat, sun, or strong light.

## WARNINGS AND PRECAUTIONS

1. This MMP-7 ELISA assay kit is designed for RESEARCH USE ONLY.
2. Please refer to the U.S. Department of Health and Human Services (Bethesda, MD, USA) publication No. (CDC) 88-8395 on laboratory safety procedures or any other local or national regulation.
3. The components in this MMP-7 ELISA assay kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting as well as following the exact time and temperature requirements prescribed is essential. Any deviation from this may yield invalid data.
5. Follow local guidelines for disposal of all waste material.

## REAGENT PREPARATION

1. Prepare 1X Wash buffer by adding contents of the 10X Wash bottle to 270ml of distilled or deionized water. Store at room temperature (18-26°C).
2. Prepare 1X Streptavidin-HRP by adding 10ml distilled or deionized water to contents of bottle and mix gently.

## ASSAY PROCEDURE

Bring all specimens and MMP-7 ELISA assay kit reagents to room temperature (18-26°C) and gently mix. If precipitate is seen in standards, vortex vigorously after reaching room temperature to re-suspend standard.

1. Thaw serum samples and bring to room temperature. Briefly vortex or mix samples.
2. Dilute serum samples 1:4 in 1X PBS before being added to the plate.
3. Dispense 50µl of MMP-7 standards, calibrators, and specimens into appropriate wells.
4. Dispense 50µl of Biotin-labeled Antibody into each well and incubate at 30°C for 1 hour at lowest rpm in shaking incubator.
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbance paper or paper towel after each wash.
6. Strike the microtiter plate sharply onto absorbance paper or paper towels to remove all residual liquid droplets.
7. Dispense 100µl of Streptavidin-HRP into each well and incubate at room temperature for 30 minutes with gentle agitation.
8. Repeat steps 5 and 6.
9. Dispense 100µl TMB Substrate into each well and incubate at room temperature in the dark for 30 minutes.
10. Stop the reaction by adding 100µl of Stop Solution into each well.
11. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
12. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

## CALIBRATOR AND STANDARD CURVE

Calibration and Standard Curve will be run in duplicate on each Microtiter Plate used for testing. Before running bring calibrators and standards to room temp and mix.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values (A450) for each set of standards, calibrators, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of MMP-7 in ng/ml from the standard curve.

**Example of Standard Curve**
Results of a typical standard run with the MMP-7 ELISA assay kit with optical density readings at 450nm shown in the Y axis against MMP-7 concentrations shown in the X axis. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

| Standard | MMP-7 values (ng/ml) | Absorbance (450nm) |
|---|---|---|
| 1 | 0 | 0.1555 |
| 2 | 4 | 0.2485 |
| 3 | 8 | 0.2730 |
| 4 | 16 | 0.4310 |
| 5 | 32 | 0.7610 |
| 6 | 64 | 1.3795 |

## CALIBRATORS
Calibrator 1 range: 7-13 ng/ml
Calibrator 2 range: 45-55 ng/ml

## EXPECTED VALUES AND SENSITIVY
The expected MMP7 values for healthy women are yet to be determined in this assay. The minimum detectable concentration of MMP-7 in this assay is estimated to be 1ng/ml.

## LIMITATIONS OF THE PROCEDURE
1. Reliable and reproducible results will be obtained when the assay procedure is carried with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Lipemia and Icteric specimens are unacceptable, may cause invalid results.

## WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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*For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*