Rat MMP10 ELISAKit (Competitive EIA)

User Manual

Catalog No. LS-F51185

SAMPLE ONLY

It is important that you read this entire manual carefully before starting your experiment.

This kit is for **Research Use Only**.
This kit is not approved for use in humans or for clinical diagnosis.

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ASSAY SPECIFICATIONS

Target:	MMP10
Synonyms:	MMP10, matrix metallopeptidase 10 (stromelysin 2), Matrix metalloproteinase-10, SL-2, STMY2, Stromelysin 2, Stromelysin-2, MMP-10, Transin 2, Transin-2, Stromelysin2, Matrix metalloprotease 10
Specificity:	This kit is for the detection of Rat MMP10. No significant cross-reactivity or interference between MMP10 and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.
Sample Types:	This kit is intended for use with samples such as Plasma, Serum, and Tissue Homogenates. It has been empirically tested using the standard supplied with the kit (typically a recombinant protein).
Detection:	Colorimetric - 450nm (TMB)
Measurement:	Quantitative
Detection Range:	80-2000 pg/ml
Sensitivity:	Typically less than 50 pg/ml
Performance:	Intra-Assay CV (<15%); Inter-Assay CV (<15%)
Limitations:	This kit is for **Research Use Only** and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis.

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Assay Principle

This assay is based on the competition ELISA principle. Each well of the supplied microtiter plate has been pre-coated with an anti-Ig-antibody. Standards or samples are added into the wells, as well as a fixed quantity of Horseradish Peroxidase (HRP)-conjugated target antigen and an antigen-specific capture antibody. The antigen-specific capture antibody binds to the anti-Ig-antibody coating the plate. The free antigen in the sample competes with the HRP-conjugated antigen for binding to the capture antibody. Unbound antigen is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contrast to typical Sandwich ELISA assays, in competition assay the greater the amount of antigen in the sample, the lower the color development and optical density reading.

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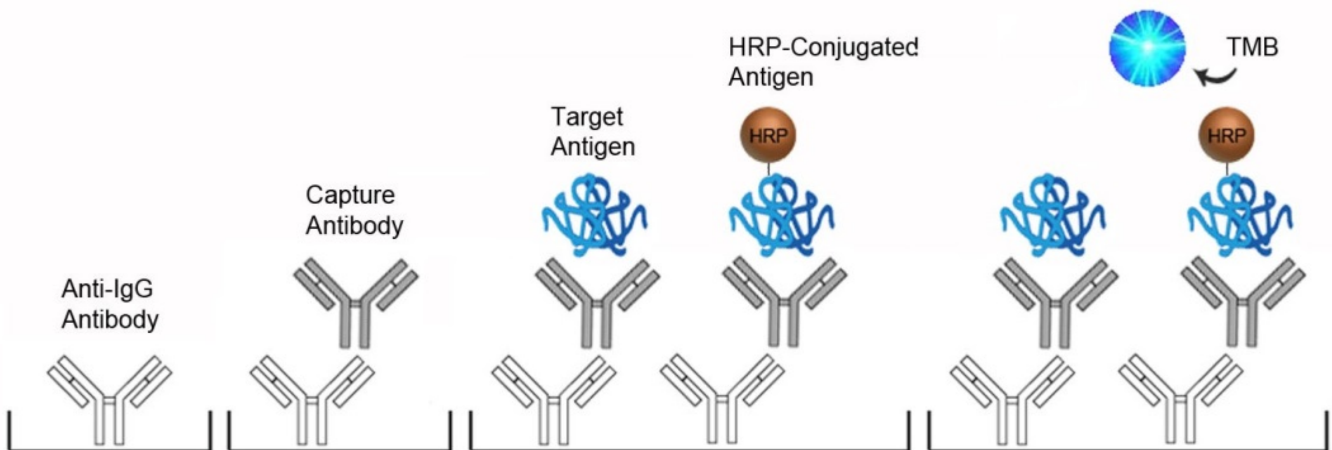
ASSAY PRINCIPLE IMAGE



KIT COMPONENTS AND STORAGE

Component	Quantity
Coated 96-well Assay Plate	1
Standards	vials x ml
Antibody	1 vial x 6 ml
HRP-conjugate	1 vial x 120 ml
Wash Buffer ()	1 vial x ml
Substrate A	1 vial x 7 ml
Substrate B	1 vial x 7 ml
Stop Solution	1 vial x ml
Adhesive Plate Sealers	4
Instruction Manual	1

KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, the kit can be stored at 2-8°C for 1 month. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

OTHER REQUIRED SUPPLIES

- Microplate reader with 450nm wavelength filterwith the correction wavelength set at 600 nm - 630 nm if available. Subtract the 600 nm - 630 nm reading from the 450nm reading to account for imperfections in the plate.
- High-precision pipette and sterile pipette tips
- Eppendorf tubes
- 37°C incubator
- Deionized or distilled water
- Absorbent paper

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Assay Planning

Before using this kit, researchers should consider the following:

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Experimental Layout and Sample Preparation).
7. Ensure that the kit is properly stored and do not use it beyond its expiration date.
8. When using multiple lots of the same kit do not substitute reagents from one kit to another. Review each manual carefully as changes can occur between lots. To control for inter-assay variability include a carry-over control sample.

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Experimental Layout

The following is an example of how to layout a study. A dilution series of the positive control Standard should be run in duplicate or triplicate, with the last well in each series being the negative control blank. Samples should also be run in duplicate or triplicate. Unknown samples should be run as a dilution series in order to identify the optimal dilution that produces an OD reading within the OD range of the positive control Standard dilution series.

Example 1: Standard Curve and dilution series of an unknown sample.

	1	2	3	4	...
A	Standard S0	Standard S0	Sample (1:1)	Sample (1:1)	...
B	Standard S1	Standard S1	Sample (1:10)	Sample (1:10)	...
C	Standard S2	Standard S2	Sample (1:100)	Sample (1:100)	...
D	Standard S3	Standard S3	Sample (1:1k)	Sample (1:1k)	...
E	Standard S4	Standard S4	Sample (1:10k)	Sample (1:10k)	...
F	Standard S5	Standard S5	Sample (1:100k)	Sample (1:100k)	...
G	Blank	Blank	Sample (1:1,000k)	Sample (1:1,000k)	...
H	Blank	Blank	Sample (1:10,000k)	Sample (1:10,000k)	...

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Example 2: Standard Curve and samples run in duplicate.

	1	2	3	4	...
A	Standard S0	Standard S0	Sample A	Sample E	...
B	Standard S1	Standard S1	Sample A	Sample E	...
C	Standard S2	Standard S2	Sample B	Sample F	...
D	Standard S3	Standard S3	Sample B	Sample F	...
E	Standard S4	Standard S4	Sample C	Sample G	...
F	Standard S5	Standard S5	Sample C	Sample G	...
G	Blank	Blank	Sample D	Sample H	...
H	Blank	Blank	Sample D	Sample H	...

Sample Collection

This assay is intended for use with samples such as Plasma, Serum, and Tissue Homogenates. The sample collection protocols below have been provided for your reference.

Breast Milk - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

Cell Lysates - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication 4 times. Alternatively freeze the cells to -20°C and thaw to room temperature 3 times. Centrifuge at 1500×g for 10 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying.

Erythrocyte Lysates - Centrifuge whole blood for 20 minutes at 1000×g to pellet the cells and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Freeze (-20°C)/thaw (room temperature) the cells 3 times. Centrifuge at 5,000×g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant for assaying. Erythrocyte lysates must be diluted with Sample Diluent before running.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying.

Platelet-Poor Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. It is recommended that samples should be centrifuged for 10 minutes at 10,000×g for complete platelet removal. Collect the supernatant for assaying.

Sperm and Seminal Plasma - Allow semen to liquefy at room temperature or 37°C. After liquefaction, centrifuge at 2,000×g for 10-15 minutes. Collect seminal plasma supernatant for assaying. Wash the precipitated protein 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication then centrifuge at 2,000×g for 10-15 minutes. Collect the supernatant for assaying.

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant for assaying.

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Tissue Homogenates - Because preparation methods for tissue homogenates vary depending upon tissue type, users should research tissue specific conditions independently. The following is one example only. Rinse tissues in PBS* to remove excess blood and weigh before homogenization. Finely mince tissues and homogenize them in 5-10mL of PBS*with a glass homogenizer on ice. Lyse the cells by ultrasonication or freeze (-20°C)/thaw (room temperature) 3 times. Centrifuge homogenate at 5000×g for 5 minutes. Collect the supernatant for assaying.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter and collect the supernatant for assaying.

Cell culture supernatants, cerebrospinal, follicular, and lung lavage fluids, saliva, sweat, tears, and other biological fluids - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying.

* 1xPBS (0.02mol/L pH7.0-7.2)

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Sample Collection Notes

1. LifeSpan recommends that samples are used immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturation, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. LifeSpan is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer-supplied samples used with the kit.

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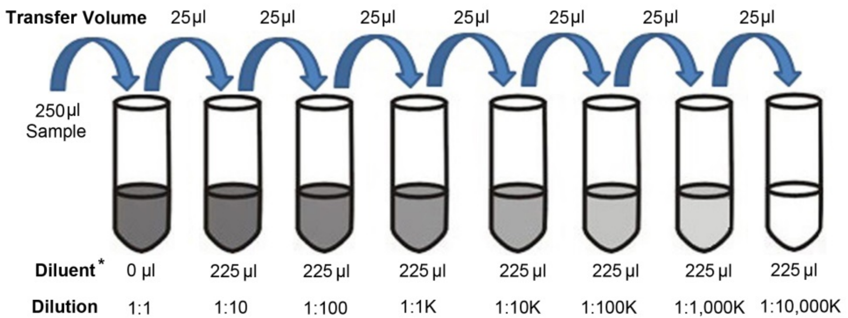
Sample Preparation

The resulting Optical Density (OD) values of your sample must fall within the OD values of the standard curve in order for the calculated antigen concentration to be accurate. In many cases samples will need to be diluted in order to lower the antigen concentration to sufficient levels. Information about antigen concentrations within various sample types may be available from the published literature; however, it is often necessary to run a dilution series of each sample type. The following will prepare sufficient volumes to run the Sample dilution series in triplicate. In the case of small volume samples the first step in the series can be a dilution, like 1:5 or 1:10, rather than undiluted sample. Running duplicate or triplicate wells for each sample is recommended.

* **Always dilute samples in the same buffer as the Standard used to generate the Standard Curve, in this case ddH$_2$0**.



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STANDARD PREPARATION

The standards for this kit are supplied pre-diluted. No additional preparation is needed. Briefly spin down tubes before opening.

Standard 0 (S0): 0 pg/ml

Standard 1 (S1): Range Low pg/ml

Standard 2 (S2): Null pg/ml

Standard 3 (S3): Null pg/ml

Standard 4 (S4): Null pg/ml

Standard 5 (S5): Range High pg/ml

REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use.

1x Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 300 ml of Working Wash Buffer by diluting 15 ml of x Wash Buffer Concentrate with 285 ml of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared.

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REAGENT PREPARATION NOTES

1. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
2. Reagents may adhere to the tube wall or cap during transport so centrifuge tubes briefly before opening.
3. All solutions should be gently mixed prior to use.
4. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10 µL is not recommended.
5. Substrate Solution is easily contaminated so sterility precautions should be taken. Substrate Solution should also be protected it from light.
6. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
7. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

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ASSAY PROCEDURE

Bring all reagents and samples to room temperature without additional heating and mixed thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents, working standards and samples as directed in the previous sections.

1. Set a Blank well without any solution.
2. Add 50 µl of **Standard** or **Sample** per well.
3. Add 50 µl of **HRP-conjugate** to each well (excluding the Blank well), then add 50 µl of **Antibody** , gently agitate to ensure thorough mixing, cover with a new plate sealer, and incubate for 60 minutes at 37°C.
4. Aspirate the liquid from each well and wash 3 times. Wash by adding approximately 200 µl of **Wash Buffer** using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 10 seconds before completely aspirating. After the last wash, aspirating remove any remaining Wash Buffer then invert the plate and tap against clean absorbent paper.
5. Add 50 µl of **Substrate A** and 50 µl **Substrate B** to each well, gently agitate to ensure thorough mixing, and incubate **in the dark** for 15 minutes at 37°C.
6. Add 50 µl of **Stop Solution** to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the substrate solution.
7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nmwith the correction wavelength set at 600 nm - 630 nm if available. Subtract the 600 nm - 630 nm reading from the 450nm reading to account for imperfections in the plate.

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ASSAY PROCEDURE NOTES

1. **ELISA Plate**: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant and stored at -20°C.
2. **Solutions**: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions:** All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing**: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing**: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time**: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance readings.
8. **Reading**: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any

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residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.

9. **Reaction Time Control**: Control reaction time should be strictly followed as outlined.
10. **Stop Solution**: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
11. **Mixing**: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable results.
12. To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

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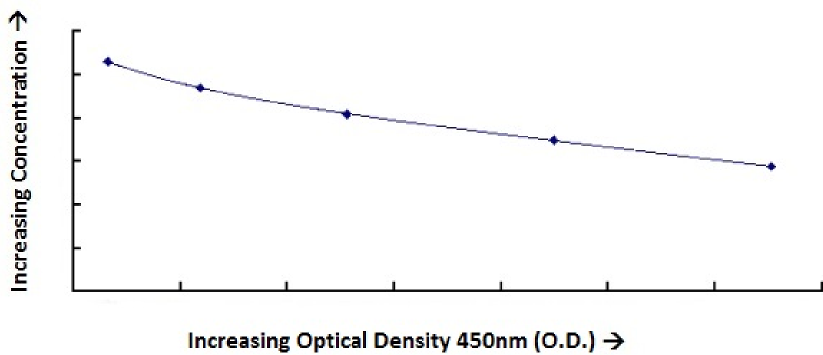
ASSAY PROCEDURE SUMMARY

Add 50 µl of **Substrate A** and 50 µl **Substrate B** to each well and incubate in the dark for _ SubstrateIncTime _ minutes at **37°C.Add 50 µl** of Substrate A and 50 µl Substrate B to each



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