Mouse Mast Cell Protease-1 (MCPT-1) Detection ELISA Kit

Catalog # 6046

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse mast cell protease-1 (MCPT-1)
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	0.8 - 50 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Mouse serum, culture medium, and biological fluids
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (3.1-6.7%)/Inter-Assay (1.7-8.0%)/Spiking Test (91-108%)
NOTES:	N/A

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION

Mast cells (MCs) are primarily recognized for their roles in allergic diseases, although they also participate in other health and disease processes. These cells accumulate at the sites of Th2 cell activation and initiate immediate hypersensitivity reactions. During IgE-associated biological responses, antigen-specific IgE antibodies bound to FcεRI on the plasma membrane of MCs undergo antigen-dependent cross-linking, leading to the secretion of biologically active products involved in allergic reactions. These products include vasoactive amines (histamines), neutral proteases (chymases), proteoglycans (heparans), and various cytokines and growth factors through a process called degranulation(1).

Mouse mast cell protease-1 (MCPT-1) is a β-chymase, a type of serine protease stored and secreted by intestinal mucosal mast cells, which are found in the intestinal epithelium (2). It shares 74% of its amino acid homology with its rat counterpart, rat mast cell protease-II (MCP-II) (3).

MCPT-1 serves as a marker for mast cell activation and degranulation. Although it is expressed constitutively and is detectable in the sera of normal mice, parasites in the gut cause systemic levels to increase dramatically within two days and peak at two weeks following infection (4). Deficiency in MCPT-1 is associated with significantly delayed expulsion of *Trichinella spiralis*, indicating its important role in the host defense against intestinal parasites (5). Elevated mouse MCPT-1 levels are also observed during intestinal allergic hypersensitivity reactions and have been reported as a marker for food allergy models (6). The mechanism is not fully understood, although it has been shown to be a tight junction-breaking protease, thus increasing the permeability of the small intestine (7).

Chondrex, Inc. provides a mouse MCPT-1 Detection ELISA kit (Cat # 6046) to determine MCP-1 levels in biological fluids and sera. This kit contains enough reagents to measure 40 samples in duplicate together with standards. Please visit www.chondrex.com or contact support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard mouse MCPT-1 (60461)	1 vial	50 ng, lyophilized	-20°C
Anti-mouse MCPT-1 Detection Antibody (60463)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Anti-mouse MCP-1 Antibody Coated ELISA Plate (Red)	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of diluted standards or samples in wells

↓ Incubate at room temperature for 2 hours.
Wash plates.

Add 100 µl of diluted Detection Antibody

↓ Incubate at room temperature for 1 hour.
Wash plates.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plates.

Add 100 µl of TMB solution

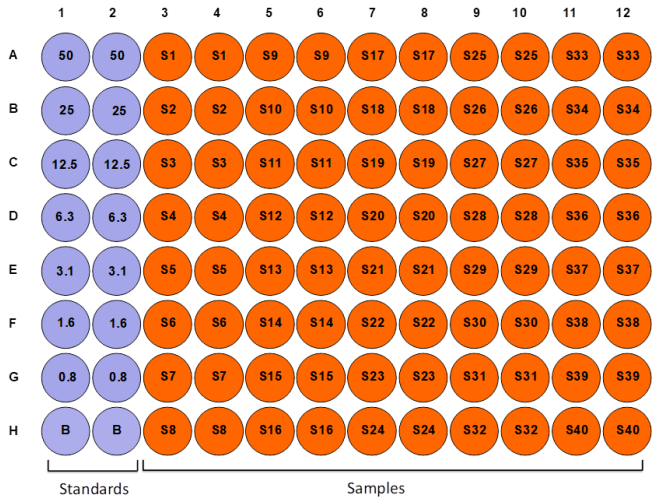
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

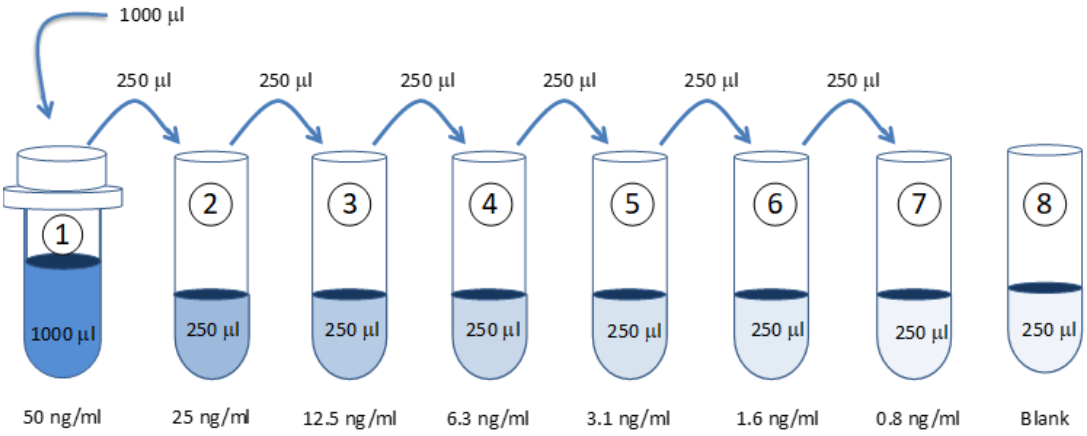
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.8 - 50 ng/ml. Dissolve one vial of Standard (50 ng/vial) in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) and keep it as a standard stock. Then serially dilute it with Solution B. For example, mix 250 µl of the 50 ng/ml solution with an equal volume of Solution B to make a 25 ng/ml solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/ml standard solutions.



2. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution B depending on the estimated mMCP-1 level in the samples. Two or three different sample dilutions are recommended if the mMCP-1 levels in the samples are unknown.

3. **Add Standards and Samples**: Add 100 µl of standards, Solution B (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

4. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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5. **Add Detection Antibody**: Dilute one vial of Detection Antibody in 10 ml Sample/Standard/Antibody Dilution buffer (Solution B). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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CALCULATING RESULTS

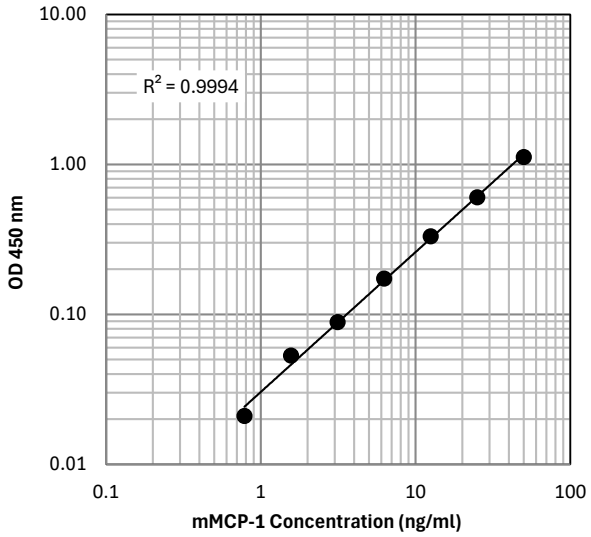
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 0.8 to 50 ng/ml.

4. The ng/ml of antigen in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the mMCP-1 concentration (ng/ml) in the original test samples.

Figure 1 - A Typical Standard Curve for the mouse MCPT-1 Detection ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for the mouse MCPT-1 Detection ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	6.7	4.4	3.1
Inter-Assay CV (%)	2.6	1.7	8.0
Spike Test* (%)	91%	108%	99%

*Known amounts of mouse MCP-1 were added to samples and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay MCPT-1 by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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