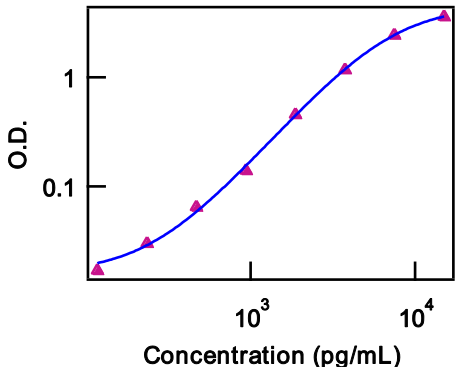
Mouse MCPT-1 (mMCP-1) ELISA Ready-SET-Go!®

Catalog Number: 88-7503
Also known as: Mouse Mast Cell Protease-1
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Mouse MCPT-1 ELISA Ready-SET-Go!®

Product Information

Contents: Mouse MCPT-1 (mMCP-1) ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7503
Sensitivity: 120 pg/mL
Standard Curve Range: 120-15,000 pg/mL

Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

This Mouse MCPT-1 Ready-SET-Go!® reagent set includes all of the necessary buffers and reagents to perform enzyme-linked immunosorbent assays. This set has been optimized for the accurate and precise measurement of mouse MCPT-1 in serum, and tissue culture supernatant samples.

MCPT-1 (Mast Cell Protease-1) is a β-chymase, a type of serine protease stored and secreted in a tissue-specific manner by mucosal mast cells. MCPT-1 is a chymotryptic protease, referring to its ability to cleave proteins and peptides after an aromatic amino acid. It shares 74% sequence homology with its rat counterpart, rat Mast Cell Protease-II (rMCPT-II) and has no direct human counterpart.

MCPT-1 is the only chymase expressed by intestinal mucosal mast cells, which are found in the intestinal epithelium. 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. It plays an important role in host defense against intestinal parasites, as mice deficient in MCPT-1 display delayed response upon infection. Elevated MCPT-1 levels are also observed during intestinal allergic hypersensitivity reactions. The mechanism of its action is not fully understood, although it is believed to increase intestinal permeability similarly to rMCPT-II, possibly by cleaving the proteins at tight junctions between cells.

Components

Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant protein for generating standard curve and calibrating samples
Coating Buffer. 10X PBS ELISA Coating Buffer
Assay Diluent. 5X Concentrated
Detection Enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Solution
Certificate of Analysis. Lot-specific instructions for the dilution of antibodies and standards
96-Well Plates. Corning Costar flat-bottom plated (included with catalog numbers ending in -22 and -86)

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

Mouse MCPT-1 (mMCP-1) ELISA Ready-SET-Go!®

Catalog Number: 88-7503
Also known as: Mouse Mast Cell Protease-1
RUO: For Research Use Only. Not for use in diagnostic procedures.

Applications Reported
This ELISA set is for the quantitative detection of mouse MCPT-1 in serum, plasma, and tissue culture supernatant samples.

Applications Tested
This assay was validated for the detection of endogenouse MCPT-1 with tissue culture supernatant collected from PMA and Ionomycin stimulated balb/c bone marrow-derived mast cells. MCPT-1 was also detected in unstimulated mast cell cultures in presence of certain growth factors, as well as serum samples taken from normal balb/c and C57 mice. These levels are lower than those in stimulated cultures. Basal levels of MCPT-1 in circulation have been reported.

This assay was tested for specificity on a panel of 72 cytokines at 100 ng/ml. No cross-reactivity was detected to any sample.

References
Graepel R, Leung G, Wang A, Villemaire M, Jirik FR, Sharkey KA, McDougall JJ, McKay, DM. Murine autoimmune arthritis is exaggerated by infection with the rat tapeworm, Hymenolepis diminuta. International Journal for Parasitology. (2013), doi: http://dx.doi.org/10.1016/j.ijpara.2013.02.006.

Caughey GH. Mast cell tryptases and chymases in inflammation and host defense. Immunol Rev. 2007 Jun; 217: 141-154.

Knight PA, Wright SH, Lawrence CE, Paterson YY, Miller HR. Delayed expulsion of the nematode Trichinell spiralis in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. J Exp Med. 2000 Dec 18; 192(12): 1849-56.

Miller HRP, Wright SH, Knight PA, Thornton EM. A novel function for Transforming Growth Factor- beta1: upregulation of the expression and the IgE-independent extracellular release of a mucosal mast cell granule-specific beta-chymase, Mouse Mast Cell Protease-1. Blood. 1999 May 15; 93(10): 3473-86.

Wastling JM, Scudamore CL, Thornton EM, Newlands GFJ, Miller HRP. Constitutive expression of mouse mast cell protease-1 in normal balb/c mice and its up-regulation during intestinal nematode infection. Immunology 1997; 90: 308-13.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

Protocol: ELISA Ready-Set-Go!

The following protocol is a general guideline for the Ready-SET-Go! Sets

Materials Provided

- Please refer to the Certificate of Analysis (C of A) for components

Other Materials Needed

- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M H_3PO_4 (recommended) or 2N H_2SO_4
- Pipettes
- Refrigerator & frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp®)
  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp (Cat. No. 44-2404) 96-well plates
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

Experimental Procedure

1. Coat Corning Costar 9018 (or Nunc Maxisorp®) ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with >250 µL/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µL/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Optional: Aspirate and wash at least once with Wash Buffer.
5. Using 1X Assay Diluent*, dilute standards as noted on the C of A to prepare the top concentration of the standard. Add 100 µL/well of top standard concentration to the appropriate

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

wells. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points. Add 100 µL/well of your samples to the appropriate wells. Seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).

6. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
7. Add 100 µL/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
9. Add 100 µL/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes**.
11. Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µL of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

NOTES:

**** Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.***

*****The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.***

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

Quick Guide: Standard Calibration

The following table indicates the protein standard contained in the Ready-SET-Go! is calibrated against NIBSC standards.

Table of Standard Calibration

Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-8	1	1.8	180	89/520
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIL-17A	1	0.9	9000	01/420
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532

* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)

ELISA Troubleshooting Guide

Problem	Possibility	Solution
A. High background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA being careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless. Replace
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat using correct dilutions
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA using the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA making no enzyme inhibitor is present in any buffers.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

	4. Coated capture antibody in Assay Diluent rather than Coating Buffer	4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody.
C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly, with a soak time.
	2. Incorrect dilutions of standard	2. Follow recommendations of standard preparation exactly as written on the C of A
	3. Insufficient incubation time	3. Repeat ELISA following the protocol carefully for each step
	4. Incorrect storage of reagents	4. Store reagents at the correct temperature asn indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards.
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see C of A. Edge effects can be avoided by moving samples and standards in from the edge of the plate.
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Reagents have expired	4. Order a new Ready-Set-Go ELISA.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com