Quantikine® ELISA

Mouse Chitinase 3-like 1 Immunoassay

Catalog Number MC3L10

For the quantitative determination of mouse Chitinase 3-like 1 (CHI3L1) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Mouse chitinase 3-like 1 (CHI3L1), also known as cartilage glycoprotein 39 (Cgp39), belongs to the glycosyl hydrolase family 18. It is a 40 kDa extracellular monomeric single chain glycoprotein containing 381 amino acids, which shares 77%, 74%, and 70% identity with that of rats, humans, and canines, respectively (1). Structurally, CHI3L1 is analogous to chitinases. Like chitinases, it is able to bind chitins with different lengths, however, it displays no chitinase activity. CHI3L1 is widely expressed by different types of cells, such as monocytes, macrophages, and chondrocytes. The physiological functions of CHI3L1 have not been fully explored. Several lines of evidence have suggested that it may participate in tissue remodeling by utilizing its chitin binding ability to modulate signal transduction pathways that are involved in extracellular matrix destruction. One observation is that CHI3L1 is induced in mammary epithelial cells a few days after weaning when the mammary gland is subjected to drastic remodeling. It is, thus, also named breast regressing protein 39 (Brp39). Other experiments have found that CHI3L1 is over-expressed in Neu/Ras oncogene-induced murine mammary gland tumors, gastric cancer, and Alzheimer's disease mouse models (2-4). Another function of CHI3L1 might be defense against pathogens. In several colitis mouse models, it is upregulated specifically in inflamed mucosa, indicating that it may play a pathogenic role by enhancing the adhesion and invasion of bacteria into colonic epithelial cells (5). In humans, CHI3L1 (also named YKL-40) has been frequently investigated as a biomarker for various pathological conditions. Microarray gene analysis has shown that CHI3L1 is one of the most over-expressed genes in glioblastoma, papillary thyroid carcinoma, and extracellular myxoid chondrosarcoma (6-8). It is also upregulated in many types of solid tumors, such as breast, colon, lung, kidney, and ovary. In cancer patients, high levels of pre-operative CHI3L1 in circulation are often associated with advanced disease stages and poor outcome (9). It has been hypothesized that CHI3L1 may contribute to cancer cell proliferation and differentiation by protecting them from undergoing apoptosis, stimulating angiogenesis, and promoting extracellular matrix remodeling. Furthermore, elevated CHI3L1 levels in serum have also been observed in some other non-malignant diseases characterized by inflammation and tissue remodeling, such as arthritis, severe bacterial infection, inflammatory bowel disease, and liver cirrhosis (10-12).

The Quantikine® Mouse Chitinase 3-like 1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse CHI3L1 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse CHI3L1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse CHI3L1. Results obtained using natural mouse CHI3L1 showed dose response curves that were parallel to the standard curves obtained using the Quantikine® mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse CHI3L1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse CHI3L1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any CHI3L1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse CHI3L1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of CHI3L1 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse CHI3L1 Microplate	893317	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse CHI3L1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse CHI3L1 Conjugate	893318	12 mL of a polyclonal antibody specific for mouse CHI3L1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse CHI3L1 Standard	893319	Recombinant mouse CHI3L1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse CHI3L1 Control	893320	Recombinant mouse CHI3L1 in a buffered protein base with preservatives, lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-21	895215	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution prior to assay. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-26 (diluted 1:4).

**See Reagent Preparation section.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse CHI3L1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

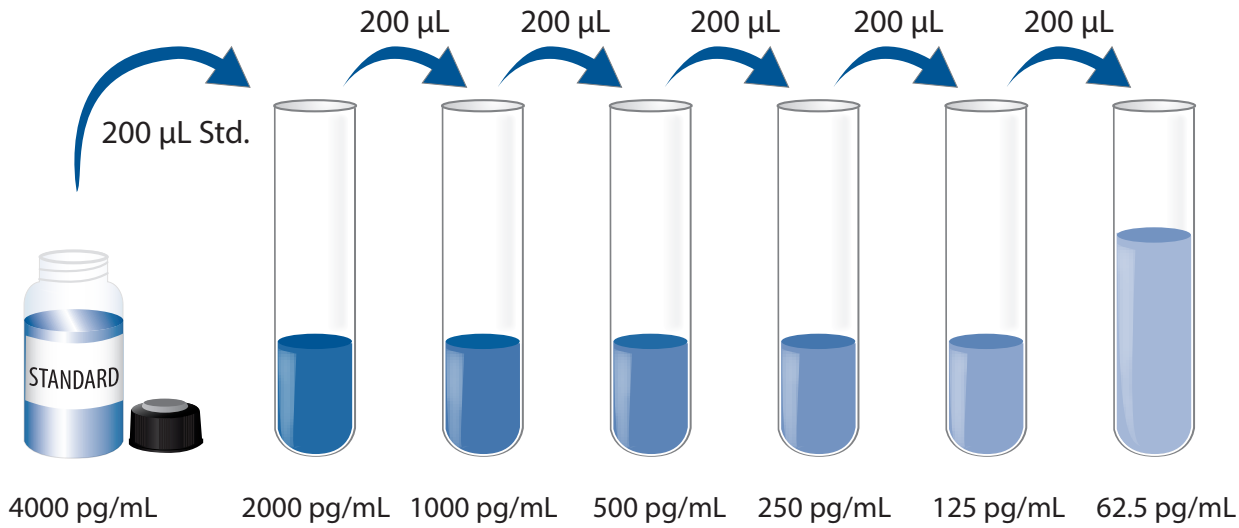
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse CHI3L1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse CHI3L1 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 4000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse CHI3L1 Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, control, and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse CHI3L1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

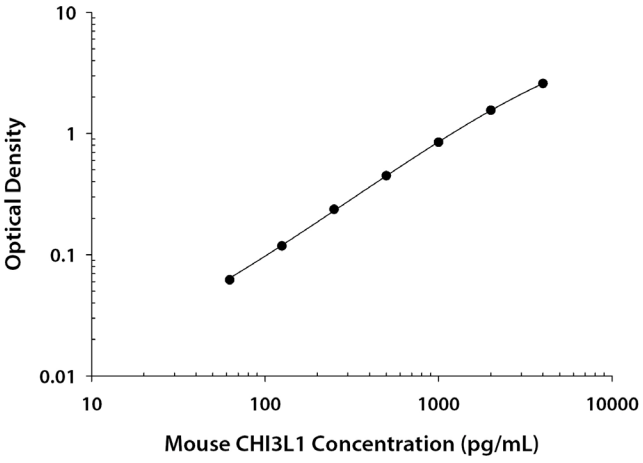
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse CHI3L1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.036	0.035	—
62.5	0.096 0.098	0.097	0.062
125	0.152 0.154	0.153	0.118
250	0.271 0.272	0.272	0.237
500	0.476 0.491	0.484	0.449
1000	0.876 0.888	0.882	0.847
2000	1.573 1.601	1.587	1.552
4000	2.609 2.627	2.618	2.583

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	187	480	1533	205	511	1530
Standard deviation	10.4	34.5	67.7	18.7	37.1	117
CV (%)	5.6	7.2	4.4	9.1	7.3	7.6

RECOVERY

The recovery of mouse CHI3L1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	99	88-107%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse CHI3L1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	96	98	96	105
	Range (%)	93-99	86-112	93-102	102-108
1:4	Average % of Expected	96	89	96	105
	Range (%)	90-99	82-100	92-102	93-111
1:8	Average % of Expected	97	91	101	104
	Range (%)	92-102	85-102	94-113	92-113
1:16	Average % of Expected	103	89	97	106
	Range (%)	97-109	84-100	91-103	91-117

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of mouse CHI3L1 ranged from 2.23-16.0 pg/mL. The mean MDD was 6.57 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse CHI3L1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse CHI3L1 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	39,374	23,540-98,000	15,474
EDTA plasma (n=20)	28,025	15,000-57,140	12,514
Heparin plasma (n=20)	41,384	22,100-87,940	15,054

Cell Culture Supernates:
Spleen tissue from two mice was homogenized and seeded into 100 mL of RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 18 hours. An aliquot of the cell culture supernate was removed, assayed for mouse CHI3L1, and measured 6851 pg/mL.

Lung tissue from two mice was homogenized and seeded into 100 mL of RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 18 hours. An aliquot of the cell culture supernate was removed, assayed for mouse CHI3L1, and measured 27,237 pg/mL.

Heart tissue from four mice was homogenized and seeded into 60 mL of RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 10 ng/mL rhIL-2, and 50 µM β-mercaptoethanol for 24 hours. An aliquot of the cell culture supernate was removed, assayed for mouse CHI3L1, and measured 532 pg/mL.

Liver tissue from one mouse was homogenized and seeded into 100 mL of RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 50 µM β-mercaptoethanol for 3 days. An aliquot of the cell culture supernate was removed, assayed for mouse CHI3L1, and measured 776 pg/mL.

Brain tissue from two mice was homogenized and seeded into 100 mL of RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 18 hours. An aliquot of the cell culture supernate was removed, assayed for mouse CHI3L1, and measured 199 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse CHI3L1.

The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range mouse CHI3L1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
Chitotriosidase/CHIT1

Recombinant human:
Chitinase 3-like 1
Chitinase 3-like 2
Chitotriosidase/CHIT1

Others:
bovine Collagen I
Heparin sodium salt
Hyaluronan

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