Quantikine® ELISA

Human Chitinase 3-like 1 Immunoassay

Catalog Number DC3L10

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

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

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Human chitinase 3-like 1 (CHI3L1) is also known as cartilage glycoprotein 39 (HCGP39), chondrex, and YKL-40 (YKL refers to the first three N-terminal amino acids and 40 denotes its molecular mass in kilodaltons). CHI3L1 is an extracellular monomeric single chain glycoprotein containing 383 amino acids (1-3). It belongs to the glycosyl hydrolase family 18 and is analogous to chitinases which is a group of enzymes capable of cleaving chitin, an abundant polysaccharide present in many organisms such as the exoskeleton of insects and walls of fungi. Like chitinases, CHI3L1 is able to bind chitins with different lengths; however, it displays no chitinase activity (4-6). CHI3L1 is expressed by many different types of cells, such as monocytes, macrophages, endothelial cells, and chondrocytes. The physiological functions of CHI3L1 have not been fully defined. In mice, it has been observed that CHI3L1 is induced in mammary epithelial cells a few days after weaning when the mammary gland is subjected to drastic remodeling and apoptosis (7). It has also been shown that CHI3L1 is able to suppress matrix metalloproteinase production, such as MMP-1, MMP-3, and MMP-13, induced by IL-1 and TNF-α (8). In addition, CHI3L1 can promote the growth of skin fibroblasts and synovial cells by itself or synergistically with IGF-I through activating the mitogen-activated protein kinase and protein kinase B signaling cascades (9). Moreover, it can facilitate vascular endothelial cell attachment, migration, and branching (10). Collectively, this experimental evidence suggests that CHI3L1 may utilize its chitin binding ability to communicate with other signal transduction pathways to modulate various physiologic processes, such as inflammation, apoptosis, tissue remodeling, cell growth, and angiogenesis.

The association of CHI3L1 with many diseases has been widely investigated. Microarray gene analysis has revealed that it is one of the most over-expressed genes in glioblastoma, papillary thyroid carcinoma, and extracellular myxoid chondrosarcoma (11-13). It is also upregulated in a number of solid tumors, such as cancer of the breast, colon, lung, kidney, and ovary. In cancer patients, serum CHI3L1 concentrations are often predictive of tumor stages, response to therapy, and prognosis (14, 15). Elevated serum CHI3L1 levels have also been observed in some conditions characterized by inflammation and tissue remodeling, such as arthritis, severe bacterial infection, inflammatory bowel disease, and liver cirrhosis (16-18). In asthma, serum CHI3L1 levels have been reported to correlate positively with the severity of the disease and adversely with lung function (19). Single nucleotide polymorphisms in the CHI3L1 gene promoter could cause increased CHI3L1 serum levels at birth and are associated with higher risk for asthma in childhood (20).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CHI3L1 has been pre-coated onto a microplate. Standards 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 human CHI3L1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CHI3L1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human CHI3L1 Microplate	893664	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human CHI3L1 Conjugate	893665	21 mL of a polyclonal antibody specific for human CHI3L1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human CHI3L1 Standard	893666	Recombinant human CHI3L1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-34	895265	11 mL of a concentrated buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow after time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric 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.
- Test tubes for dilution of standards and samples.
- Human CHI3L1 Controls (optional; R&D Systems®, Catalog # QC63).

PRECAUTIONS

CHI3L1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples may require dilution.

Serum and plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of human CHI3L1 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

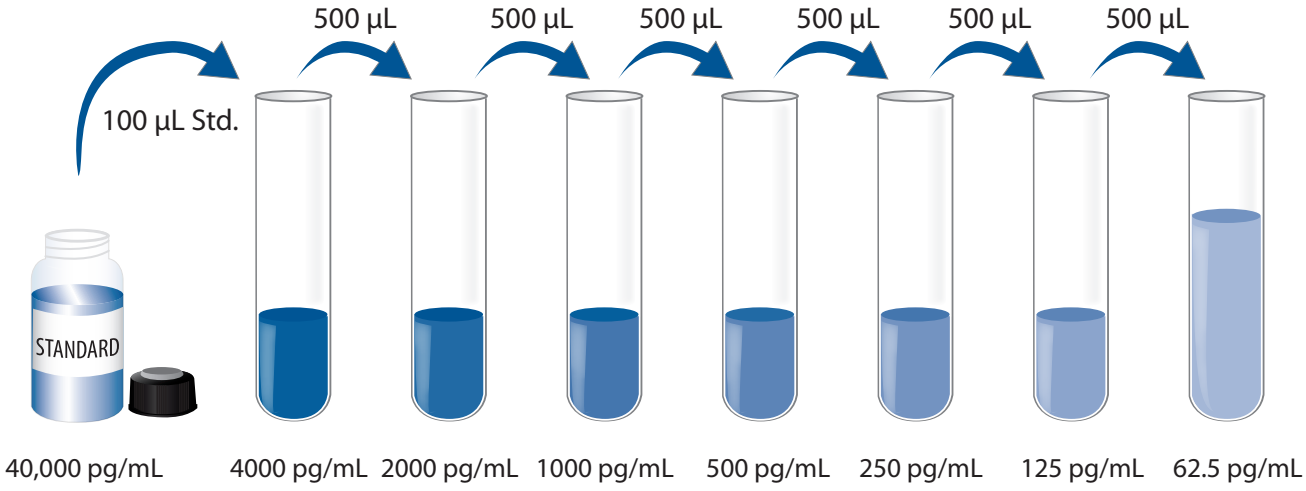
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human CHI3L1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CHI3L1 Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 40,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 4000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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 standards, samples, and controls be assayed in duplicate.

Note: *High concentrations of human CHI3L1 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, samples, and working standards 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 100 µL of Assay Diluent RD1-34 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 three times for a total of four 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 toweling.
6. Add 200 µL of Human 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 200 µL of Substrate Solution to each well. Incubate 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, 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

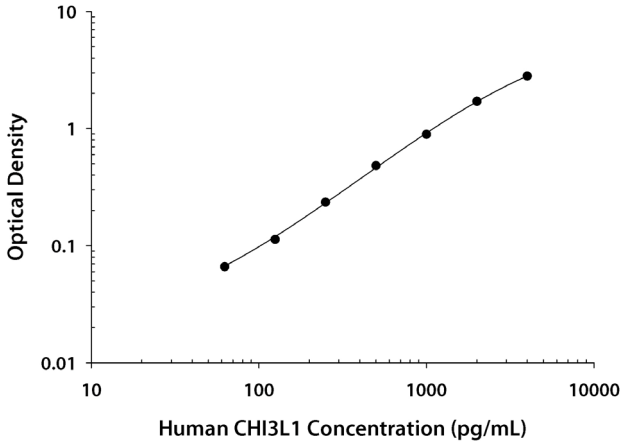
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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 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	0.009 0.010	0.010	—
62.5	0.075 0.077	0.076	0.066
125	0.122 0.123	0.123	0.113
250	0.240 0.250	0.245	0.235
500	0.469 0.514	0.492	0.482
1000	0.897 0.908	0.903	0.893
2000	1.669 1.766	1.718	1.708
4000	2.797 2.836	2.817	2.807

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)	566	1031	2202	606	1019	2212
Standard deviation	26.7	44.4	103	32.1	59.1	153
CV (%)	4.7	4.3	4.7	5.3	5.8	6.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	93-100%
Urine (n=4)	105	90-115%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human CHI3L1 were serially 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)	Urine (n=4)
1:2	Average % of Expected	96	102	101	101	101
	Range (%)	93-100	98-106	98-105	97-106	89-112
1:4	Average % of Expected	96	100	103	103	103
	Range (%)	89-100	94-106	102-103	96-109	85-111
1:8	Average % of Expected	97	101	102	105	102
	Range (%)	89-107	92-106	101-105	95-112	86-114
1:16	Average % of Expected	102	106	97	95	100
	Range (%)	88-113	103-111	93-102	94-103	86-111

*Samples were diluted prior to assay.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human CHI3L1 ranged from 1.25-8.15 pg/mL. The mean MDD was 3.55 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 human CHI3L1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human CHI3L1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	36.0	15.9 - 93.5	20.6
EDTA plasma (n=36)	27.7	10.9 - 79.1	17.5
Heparin plasma (n=36)	26.8	10.7 - 72.1	15.7
Urine (n=11)	0.64	0.14 - 1.82	0.49

Cell Culture Supernates:
HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human CHI3L1, and measured 0.944 ng/mL.

MG-63 human osteosarcoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 4 days. An aliquot of the cell culture supernate was removed, assayed for human CHI3L1, and measured 3.04 ng/mL.

U-87 MG human glioblastoma/astrocytoma cells and SK-Mel-28 human malignant melanoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 1 mM sodium pyruvate, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of each cell culture supernate was removed and assayed for human CHI3L1. U-87 MG cells measured 16.1 ng/mL, and the SK-Mel-28 cells measured 0.071 ng/mL.

HASMC human aortic smooth muscle cells were cultured in Medium 231 supplemented with smooth muscle growth supplement until confluent. An aliquot of the cell culture supernate was removed, assayed for human CHI3L1, and measured 0.623 ng/mL.

BUD-8 human fibroblasts were cultured in 90% MEM with non-essential amino acids supplemented with 10% fetal bovine serum and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for human CHI3L1, and measured 4.31 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human CHI3L1.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range human CHI3L1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Natural proteins:
Aggrecan	CHI3L1	bovine Collagen
CHI3L2	CHIT1	
CHIT1	COL13A1	

REFERENCES

1. Johansen, J.S. *et al.* (1992) J. Bone Miner. Res. **7**:501.
2. Harvey, S. *et al.* (1993) Clin. Chem. **44**:509.
3. Rehli, M. *et al.* (1997) Genomics **43**:221.
4. Hakala, B.E. *et al.* (1993) J. Biol. Chem. **268**:25803.
5. Renkema, G.H. *et al.* (1998) Eur. J. Biochem. **251**:504.
6. Fusetti, F. *et al.* (2003) J. Biol. Chem. **278**:37753.
7. Morrison, B.W. and P. Leder (1994) Oncogene **9**:3417.
8. Ling, H. and A.D. Recklies (2004) Biochem. J. **380**:651.
9. Recklies, A.D. *et al.* (2002) Biochem. J. **365**:119.
10. Malinda, K.M. *et al.* (1999) Exp. Cell Res. **250**:168.
11. Tanwar, M.K. *et al.* (2002) Cancer Res. **62**:4364.
12. Huang, Y. *et al.* (2001) Proc. Natl. Acad. Sci. USA **98**:15044.
13. Sjogren, H. *et al.* (2003) Am. J. Pathol. **162**:781.
14. Dupont, J. *et al.* (2004) J. Clin. Oncol. **22**:3330.
15. Schmidt, H. *et al.* (2006) J. Clin. Oncol. **24**:798.
16. Johansen, J.S. *et al.* (1999) Rheumatology **38**:618.
17. Vind, I. *et al.* (2003) Scand. J. Gastroenterol. **38**:599.
18. Tran, A. *et al.* (2000) Eur. J. Gastroenterol. Hepatol. **12**:989.
19. Chupp, G.L. *et al.* (2007) N. Engl. J. Med. **357**:2016.
20. Ober, C. *et al.* (2008) N. Engl. J. Med. **358**:1682.