ELISA Kit for Measuring Human Chitotriosidase

CircuLex Human Chitotriosidase ELISA Kit

Cat# CY-8074

Intended Use

The MBL Research Product **CircuLex Human Chitotriosidase ELISA Kit** is used for the quantitative measurement of human chitotriosidase in serum.

Individual users should determine appropriate conditions when using other types of samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Do not expose reagents to excessive light.

Introduction

Chitotriosidase was discovered in plasma of patients suffering from Gaucher's disease; it was found that the 1,000-fold-elevated enzyme originates from lipid-laden macrophages that accumulate in various tissues of Gaucher's patients (1). Chitotriosidase has subsequently been purified from the spleen of a Gaucher's patient and its cDNA was cloned from a human macrophages cDNA library (2, 3). This enzyme is a human chitinase member of family 18 glycosyl hydrolases (3–5), and has the capability to hydrolyze chitin. This enzyme is selectively expressed in activated tissue macrophages that accumulate in various tissues of several lysosomal diseases (6). Therefore its activity has been proposed as a biochemical marker of macrophage accumulation in Gaucher's disease (1, 7). In some other inherited lysosomal storage disorders, especially sphingolipidoses such as Niemann Pick, GM1-gangliosidosis, and Krabbe disease, which involve accumulation of different lipids, more modest elevations in plasma chitotriosidase have been noted (7). Chitotriosidase is the only biomarker identified up to date for the monitoring the efficacy of the extremely costly enzyme-replacement therapy of Gaucher patients and male Fabry patients (8).

Elevated levels of serum chitotriosidase were also found in disorders caused by the abnormal activation of immune system, including sarcoidosis (9) and atherosclerosis (10, 11). It has been shown that chitotriosidase activity was elevated up to 55-fold in extracts of atherosclerotic tissue, showing a clear connection between chitotriosidase expression and lipid-laden macrophages inside human atherosclerotic vessel wall (10). Human chitotriosidase also associates with pathogen-driven diseases, and in particular with fungal infections, suggesting the role of this enzyme in host defense against chitin-containing pathogens (12, 13). Other clinical data for instance show that chitotriosidase activity is raised in plasma of African children infected with acute Plasmodium falciparum malaria (14). Additional evidence for a role of chitotriosidase during immunological responses is the observation that the enzyme is shortly and acutely up-regulated both at the level of mRNA and activity following stimulation with prolactin, IFN-γ, TNF α and LPS, but not with IL-10 (15, 16).

In the blood stream, tissue macrophages largely secrete newly synthesized 50-kDa chitotriosidase, but about one-third is directly routed to lysosomes and proteolytically processed to the 39-kDa unit that remains catalytically active (17). A common chitotriosidase gene polymorphism leads to a null allele and therefore a defective enzyme activity. In white populations, 30% to 40% of individuals are carriers of this abnormal chitotriosidase allele and approximately 6% are homozygous (1, 18).

Principle of the Assay

The MBL Research Product **CircuLex Human Chitotriosidase ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human chitotriosidase has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human chitotriosidase present. After washing away any unbound substances, an HRP conjugated antibody specific for human chitotriosidase is added to the wells. Following a wash to remove any unbound HRP conjugated antibody, the remaining conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human chitotriosidase. A standard curve is constructed by plotting absorbance values versus human chitotriosidase concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

The MBL Research Product **CircuLex Human Chitotriosidase ELISA Kit** is designed to measure the concentration of human chitotriosidase in serum.

Summary of Procedure

Add 100 µL of diluted samples to wells
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-human chitotriosidase antibody
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓ Incubate for 10-20 minutes at room temp.
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-human chitotriosidase antibody as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing Tween®-20.

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for reconstitution of Human Chitotriosidase Standard and sample dilution. Ready to use.

Human Chitotriosidase Standard: One vial containing X* ng of lyophilized recombinant human chitotriosidase
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human chitotriosidase antibody. Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder**.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residues from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Sample Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for 60 ± 30 minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x *g*. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Other biological samples: MBL has not tested.
(*e.g.* Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles. Individual users should determine appropriate conditions when using other types of samples.)

Detailed Protocol

The MBL Research Product **CircuLex Human Chitotriosidase ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Human Chitotriosidase Standard within the kit, should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer** and **Human Chitotriosidase Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water (**ddH_2O**). Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Reconstitute **Human Chitotriosidase Standard** with X* **µL** of **Dilution Buffer** by gently mixing. After reconstitution, immediately dispense it in small aliquots (e.g. 100 µL) to plastic micro-centrifuge tubes and store below -70°C to avoid non-specific adsorption to glass surface and multiple freeze-thaw cycles. The concentration of the reconstituted Human Chitotriosidase Standard should be **36 ng/mL**, which is referred to as the **Master Standard** of human chitotriosidase.
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

Prepare Standard Solutions as follows:
Use **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. **Std.1 (3,600 pg/mL)** serves as the highest standard. **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	60 µL of Master Standard	540 µL	3,600 pg/mL
Std.2	300 µL of Std. 1 (3,600 pg/mL)	300 µL	1,800 pg/mL
Std.3	300 µL of Std. 2 (1,800 pg/mL)	300 µL	900 pg/mL
Std.4	300 µL of Std. 3 (900 pg/mL)	300 µL	450 pg/mL
Std.5	300 µL of Std. 4 (450 pg/mL)	300 µL	225 pg/mL
Std.6	300 µL of Std. 5 (225 pg/mL)	300 µL	112.5 pg/mL
Std.7	300 µL of Std. 6 (112.5 pg/mL)	300 µL	56.25 pg/mL
Blank	-	300 µL	0 ng/mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with **Dilution Buffer** before dispensing. Discard any unused Standard Solutions after use.

Sample Preparation

Dilute samples with **Dilution Buffer**.
• Serum samples may require a 50-fold dilution.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4 °C.

2. Dilute samples with **Dilution Buffer**. (See "Sample Preparation" above.)

3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.

4. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

6. Add **100 µL** of **HRP conjugated Detection Antibody** into each well.

7. Incubate the plate **at room temperature (ca.25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microplate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4 °C immediately after the necessary volume is removed.

10. Incubate the plate **at room temperature (ca.25°C) for 10-20 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below than 20 °C.

11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: 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.

Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.25 units for the blank (zero concentration), or 3.0 units for the highest standard concentration.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine the concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control and sample, and subtract the optical density of the average zero standard. Plot the optical density versus the concentration of standards and draw the best curve. Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve fits best to a sigmoidal four-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a four-parameter logistic function.

A standard curve is also to be constructed by plotting the absorbance (Y) versus log of the known concentration (X) of standards, using a cubic function. Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of optical density (Y) is plotted versus log of the known concentration (X) of standards). To determine the concentration of each sample, first find the optical density on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.

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

Measurement Range

The measurement range is 56.25 pg/mL to 3,600 pg/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human chitotriosidase concentration.

Troubleshooting

1. All samples and standards should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.

3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex Human Chitotriosidase ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date.

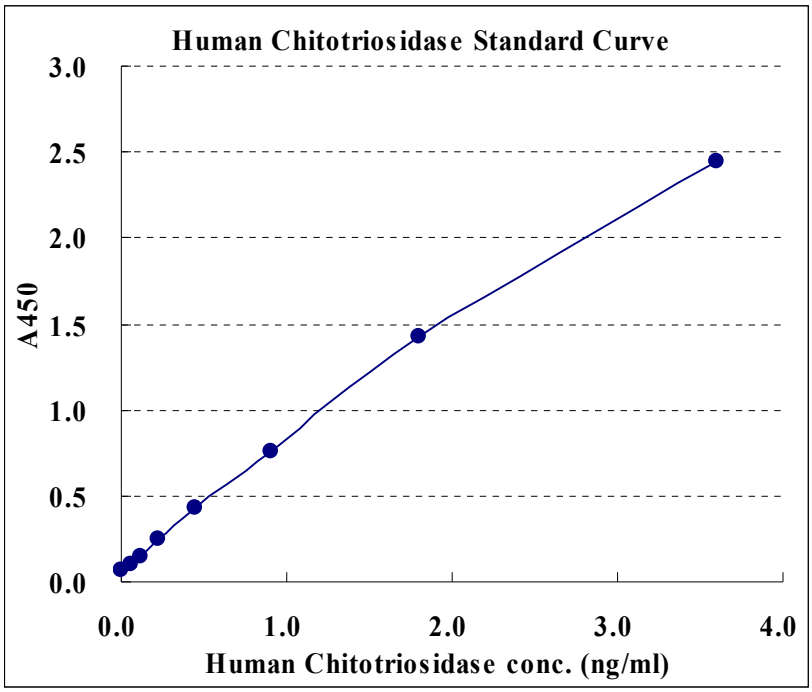
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human chitotriosidase giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 48.3 pg/ml of sample.

* Dilution Buffer was pipetted into blank wells.

Typical Standard Curve



2. Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.

• Intra-assay (Within-Run, n=16) CV=2.1-2.6 %

Human Chitotriosidase conc. (ng/ml)

	Serum 1	Serum 2	Serum 3
1	19.01	10.46	5.95
2	18.31	10.04	5.60
3	18.39	10.32	5.72
4	18.46	10.52	5.61
5	18.62	10.44	5.61
6	18.51	9.96	5.64
7	18.31	9.98	5.58
8	17.72	9.86	5.74
9	18.38	10.60	5.57
10	17.89	10.23	5.54
11	18.71	10.18	5.57
12	18.85	10.59	5.62
13	18.57	10.41	5.73
14	18.34	9.80	5.47
15	18.10	10.04	5.69
16	17.64	10.30	5.47
MAX.	**19.01**	**10.60**	**5.95**
MIN.	**17.64**	**9.80**	**5.47**
MEAN	**18.36**	**10.23**	**5.63**
S.D.	**0.38**	**0.26**	**0.12**
C.V.	**2.1%**	**2.6%**	**2.1%**

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in five separate assays to assess inter-assay precision.

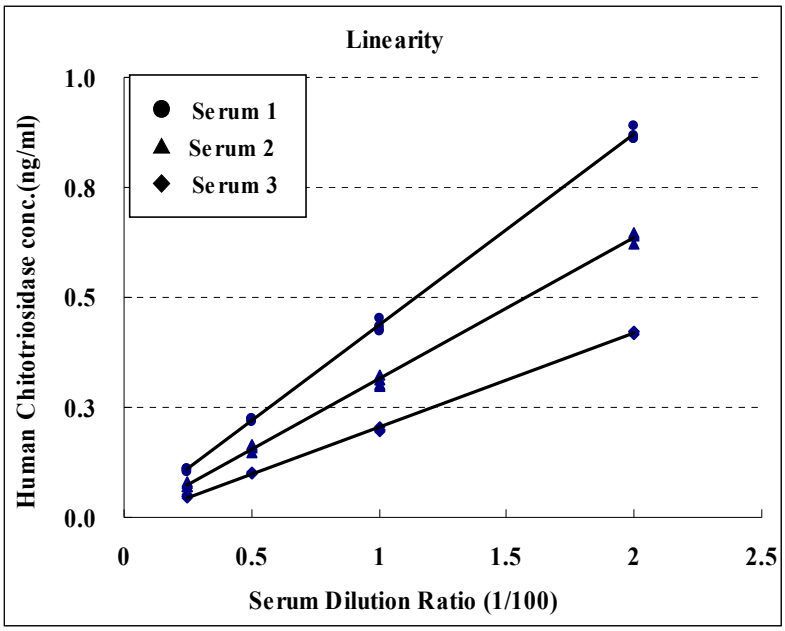
• Inter-assay (Run-to-Run, n=5) CV=3.07 - 5.02 %

Hu Chitotriosidase conc. (ng/ml)

	Serum 1	Serum 2	Serum 3
1	33.9	19.6	9.9
2	35.0	19.6	9.7
3	32.4	20.5	9.9
4	32.7	20.4	10.5
5	36.6	21.2	10.0
MAX.	**36.6**	**21.2**	**10.5**
MIN.	**32.4**	**19.6**	**9.7**
MEAN	**34.1**	**20.3**	**10.0**
S.D.	**1.71**	**0.70**	**0.31**
C.V.	**5.02%**	**3.47%**	**3.07%**

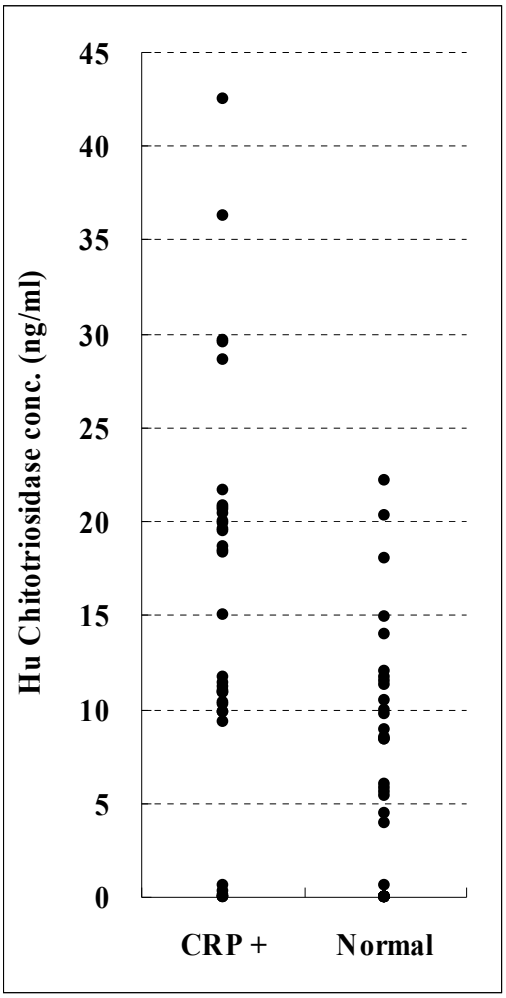
3. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of chitotriosidase were serially diluted with the Dilution Buffer to produce samples with values within the dynamic range of the assay.



Example of Test Results

Fig.1 Chitotriosidase concentrations in healthy Japanese volunteers sera (n=36) and CRP positive sera (n=35)



References

1. Hollak CE *et al*. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Invest. 1994 Mar;93(3):1288-92.

2. Renkema GH *et al*. Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. J Biol Chem. 1995 Feb 3;270(5):2198-202.

3. Boot RG *et al*. Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem. 1995 Nov 3;270(44):26252-6.

4. Hakala BE *et al*. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem. 1993 Dec 5;268(34):25803-10.

5. Renkema GH *et al*. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. Eur J Biochem. 1998 Jan 15;251(1-2):504-9.

6. Aerts JM and Hollak CE. Plasma and metabolic abnormalities in Gaucher's disease. Baillieres Clin Haematol. 1997 Dec;10(4):691-709.

7. Guo Y *et al*. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. J Inherit Metab Dis. 1995;18(6):717-22.

8. Vedder AC *et al*. Plasma chitotriosidase in male Fabry patients: a marker for monitoring lipid-laden macrophages and their correction by enzyme replacement therapy. Mol Genet Metab. 2006 Nov;89(3):239-44.

9. Grosso S *et al*. Serum levels of chitotriosidase as a marker of disease activity and clinical stage in sarcoidosis. Scand J Clin Lab Invest. 2004;64(1):57-62.

10. Boot RG *et al*. Strong induction of members of the chitinase family of proteins in atherosclerosis: chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol. 1999 Mar;19(3):687-94.

11. Artieda M *et al*. Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. Arterioscler Thromb Vasc Biol. 2003 Sep 1;23(9):1645-52.

12. Labadaridis J *et al*. Serial chitotriosidase activity estimations in neonatal systemic candidiasis. Acta Paediatr. 1998 May;87(5):605.

13. Labadaridis I *et al*. Chitotriosidase in neonates with fungal and bacterial infections. Arch Dis Child Fetal Neonatal Ed. 2005 Nov;90(6):F531-2.

14. Barone R *et al*. Plasma chitotriosidase activity in acute Plasmodium falciparum malaria. Clin Chim Acta. 2003 May;331(1-2):79-85.

15. Di Rosa M *et al*. Effect of interferon-gamma, interleukin-10, lipopolysaccharide and tumor necrosis factor-alpha on chitotriosidase synthesis in human macrophages. Clin Chem Lab Med. 2005;43(5):499-502.

16. Malaguarnera L *et al*. Prolactin induces chitotriosidase gene expression in human monocyte-derived macrophages. Immunol Lett. 2004 Jun 15;94(1-2):57-63.

17. Renkema GH *et al*. Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase. Eur J Biochem. 1997 Mar 1;244(2):279-85.

18. Eiberg H and Den Tandt WR. Assignment of human plasma methylumbelliferyl-tetra-N-acetylchitotetraoside hydrolase or chitinase to chromosome 1q by a linkage study. Hum Genet. 1997 Dec;101(2):205-7.

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