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Product Manual

Human Leukocyte cell-derived chemotaxin-2 (LECT2) ELISA Kit

Catalog No.: abx250628

Size: 96T

Range: 0.156-10 ng/ml

Sensitivity: < 0.094 ng/ml

Storage and Expiration: Store at 4°C for up to 6 months.

Application: For quantitative detection of LECT2 in Human serum, plasma, tissue homogenates or any biological fluids.

Introduction

Leukocyte cell-derived chemotaxin-2 is a protein that in humans is encoded by the LECT2 gene, and causes Lect2 amyloidosis. This gene encodes a secreted, 16 kDa protein that acts as a chemotactic factor to neutrophils and stimulates the growth of chondrocytes and osteoblasts. This protein has high sequence similarity to the chondromodulin repeat regions of the chicken myb-induced myeloid 1 protein. A polymorphism in this gene may be associated with rheumatoid arthritis.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. LECT2 antibody is pre-coated onto 96-well plates. Biotin conjugated LECT2 monoclonal antibody is used as a detection antibody. The standards, test samples and biotin conjugated detection antibody are added to the wells and washed with wash buffer. HRP Streptavidin is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The density of yellow is proportional to the LECT2 amount of sample captured in plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of LECT2 can be calculated.

Kit components

1. One 96-well plate pre-coated with LECT2 antibody
2. Lyophilized standards: 2 tubes
3. Sample / Standard diluent buffer: 20ml
4. Biotin conjugated antibody (Concentrated): 60 µl. Dilution: 1:100
5. Antibody diluent buffer: 10 ml
6. HRP Streptavidin Conjugate (SABC) (Concentrated): 120 µl. Dilution: 1:100
7. SABC diluent buffer: 10 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Pipettes and disposable pipette tips
4. Automated plate washer (optional)
5. ELISA shaker
6. 1.5 ml tubes
7. Plate cover
8. Absorbent filter papers
9. Plastic or glass container with volume greater than 1L

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Protocol

A. Preparation of sample and reagents

1. Sample
Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

✧ **Tissue lysate, body fluids and cell culture supernatants:** Centrifuge to remove particulates, assay immediately or aliquot and store at -20°C.

✧ **Serum:** Allow the serum to clot in a serum separator tube at room temperature (30 min). Centrifuge at approximately 1000 × g for 15 minutes. Analyze the serum immediately or aliquot and store at -20°C.

✧ **Plasma** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C.

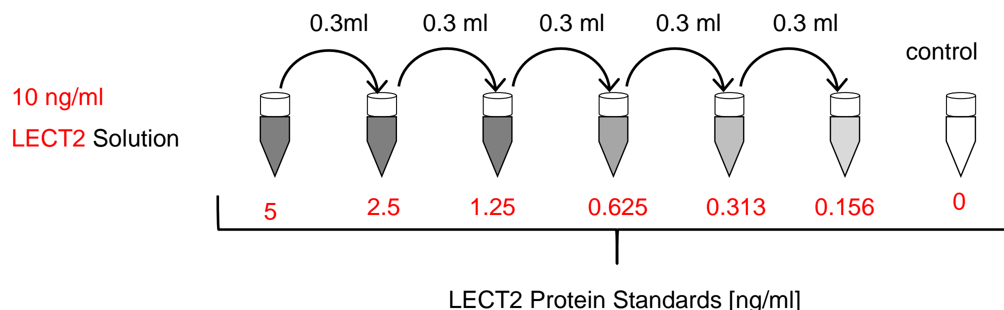
Note:

- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.

General Sample guideline:
Estimate the concentration of the target protein in the sample and select the correct dilution factor to make the diluted target protein concentration fall near the middle of the kit's range. For high concentration, dilute 1:100, for medium concentration, dilute 1:10 and for low concentration, dilute 1:2. Very low concentrations do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer
Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. add 30ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard
Reconstitution of the Lyophilized standard: standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment.
(Note: Do not dilute the standard directly in the plate)
a. 10 ng/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.
b. 5 ng/ml → 0.156 ng/ml of standard solutions: Label 6 tubes with 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.313 ng/ml, 0.156 ng/ml, respectively. Aliquot **0.3 ml** of the Sample / Standard diluent buffer into each tube. Add **0.3 ml** of the above 10 ng/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be used within 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hours before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of HRP Streptavidin Conjugate (SABC) working solution: prepare no more than 1 hour before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

B. Assay procedure

Equilibrate the SABC working solution and TMB substrate for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively and record their positions. It is recommended to measure each standard and sample in duplicate. Wash the plate before adding standard, samples and buffers.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the contents by clapping the plate on absorbent filter papers or any other absorbent material. Do not wash the plate and do NOT let the wells dry out completely at any time.
7. Add 100 µl of Biotin conjugated antibody into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and

incubate at 37°C for 60 minutes.

8. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

   Please note: For automated washing, discard the solution and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

9. Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
11. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-30 minutes. The incubation time is for reference use only, the optimal time should be determined by end user. Please do not exceed 30 min.
12. Add 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.
13. Read the O.D. absorbance at 450 nm in a microplate reader within 30' of adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human LECT2 concentration of the samples can be extrapolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from extrapolation to obtain the concentration before dilution.

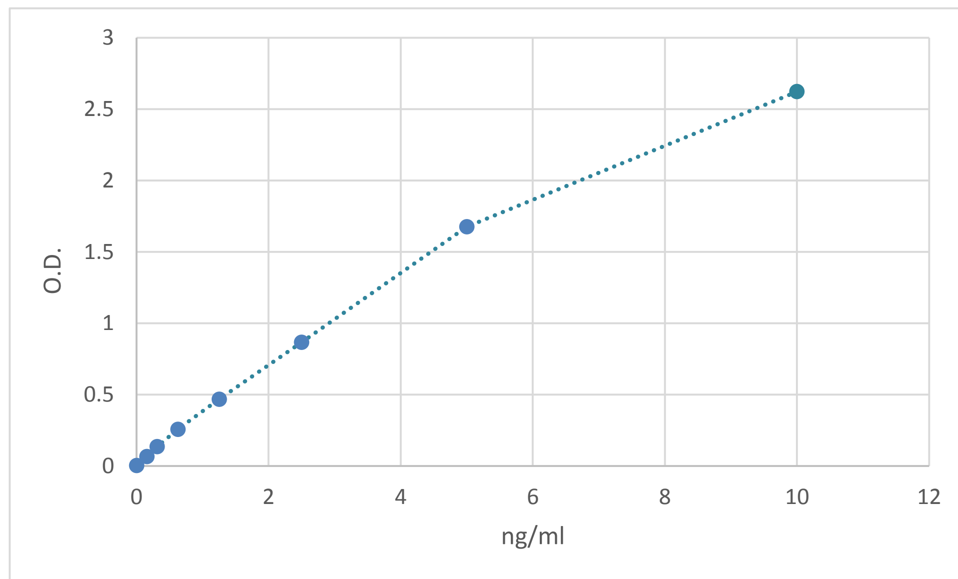
C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. It is recommended to assay all standards, controls and sample in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. To avoid cross contamination do not reuse pipette tips and tubes.
7. Do not use components from a different kit or expired ones.
8. The TMB substrate is light sensitive and should be protected from direct sunlight and UV sources. Unreacted substrate should be colorless or very light yellow in appearance. The product should be allowed to equilibrate to room temperature (25°C) prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Typical Data & Standard Curve

Typical Standard Curve- Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

ng/ml	0	0.156	0.313	0.625	1.25	2.5	5	10
OD450	0.005	0.067	0.137	0.258	0.469	0.868	1.677	2.623



This diagram is for reference only.