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SKU IP200015
LT5106TEKKBA

ELISA KIT FOR HUMAN IL-4

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.2B

INTENDED USE

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Interleukin 4 (IL4) in human serum, plasma, cell culture supernates and other biological fluids.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Human IL-4 Standard [2 vial (lyophilized)]
Sample Diluent [7mL (5x)]
Assay Buffer [7mL (5x)]
Human IL-4 Detect Antibody [120µL (100x)]
Streptavidin-HRP [120µL (100x)]
20X Wash solution [50mL]
TMB (HRP Substrate) [10mL]
Stop Solution [10mL]
Plate Sealing Membrane [2pcs]
User manual [1pc]

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.

NOT INCLUDED

Distilled Water | Pipettes and Tips | ELISA Reader and 37°C Incubator

SUMMARY AND PRINCIPLE

IL-4 is a 129 amino acids long secreted protein. IL-4 is a cytokine produced mostly by activated T lymphocytes, mast cells and basophils. It has variety of immune response modulating functions by acting on number of cell types. IL-4 participates in several B-cell activation processes.

Human IL-4 ELISA Kit employs a two-site sandwich ELISA to quantitate Human IL4 in samples. An antibody specific for Human IL4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Human IL4 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Human IL4 is added to the wells. After washing, proprietary Streptavidin-HRP conjugate is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human IL4 bound in the initial step. The color development is stopped by Stop Solution and the intensity of the color is measured.

CHARACTERISTICS

Sample Type: Serum, plasma, cell culture supernates and other biological fluids.
Detection Range: 3.125 pg/mL - 200 pg/mL
Minimum Detection Dose: 1.5 pg/mL
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%
Linearity with spiked analytes: ρ=0.99
Recovery Range: 98-116% with mean of 106% with spiked samples
Cross Reactivity: None observed with analogues.

Validity: 1 year from the date on manufacture
Storage: +2-8°C
Once opened unused wells should be put into the sealed bag with a desiccant pack and may be used for up to 1 month.

SAMPLE COLLECTION

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Cell Culture Supernates and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note:
1. Samples to be used within 24 hours may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.
4. Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

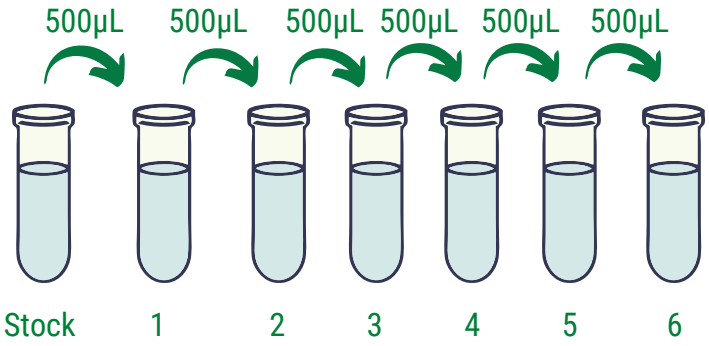
Note: The dilution of Wash Buffer and Sample Diluent/ Assay Buffer in the following operation steps is based on 96T kit. If a 48T kit is used, please scale it down.

Wash Buffer - Pour entire contents (50mL) of the Wash Buffer (20×) into a clean 1000mL graduated cylinder. Bring to final volume of 1000mL with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25˚C. Please note that Wash Buffer (1×) is stable for 30 days.

Sample Diluent/ Assay Buffer - Pour the entire contents (7mL) of the Diluent (5×) into a clean 100mL graduated cylinder. Bring to final volume of 35mL with distilled water. Mix gently to avoid foaming. Store at 2 to 8˚C. Please note that the Diluent (1×) is stable for 30 days.

HRP Substrate - The reagents should be ready within 15 minutes of use. Protect from light. 100µL of the solution is required per well.

Standard - - Reconstitute the Human IL4 standard in 1mL of Sample Diluent for a concentration of 200pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Add 500µL of Sample Diluent to each of 6 tubes labeled 100, 50, 25, 12.5, 6.25 and 3.125 pg/mL of Human IL4 standard just as below.



Concentrations
Stock- 200pg/mL
1- 100pg/mL
2- 50pg/mL
3- 25pg/mL
4- 12.5pg/mL
5- 6.25pg/mL
6- 3.125pg/mL

Human IL4 Detect Antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detect antibody should be used within 30 minutes after dilution.

Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used within 30 minutes after dilution.

Sample Diluent - If your samples need to be diluted, Sample diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernates.

PROCEDURE

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

1. Prepare all reagents 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 diluted standard and sample per well. Add 100 µL Sample Diluent to Blank well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.

4. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. Complete removal of liquid at each step is essential for 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.

5. Add 100 µL of diluted Human IL4 detect antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.

8. Repeat the aspiration/wash process for five times as in step 4.

9. Add 100 µL of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

10. 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.

11. 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.

CALCULATION

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 concentration on the x-axis against the mean absorbance for each standard on the y-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 IL4 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.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

Still if you have any concerns please feel free to write to us at customerservice@lifetechindia.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources. 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.

- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

- 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.

- Stop Solution contains 2N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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