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ELISA KIT FOR CHICKEN IL4

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

1800-120-2434
CUSTOMERSERVICE@LIFETECHINDIA.COM

VERSION 1.2B

INTENDED USE

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

KIT CONTENTS

Precoated Microplate [12 strips X 8 wells]
Standard (lyophilized) [2 vials]
Standard Diluent Buffer [20mL]
Biotinylated Antibody (100×) [120µL]
Biotinylated Antibody Diluent [12mL]
Streptavidin-HRP (100×) [120µL]
HRP Diluent [12mL]
Wash Buffer (25×) [20mL]
TMB Substrate Solution [9mL]
Stop Reagent [6mL]
Plate Covers [2 pcs]
User manual [1pc]

Note: *Store all the components at 4°C.*

MATERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37°C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

SUMMARY AND PRINCIPLE

The microtiter plate wells provided in this kit have been pre-coated with an antibody specific to chicken IL4. Standards or samples are added to the appropriate wells, then incubated with biotin-conjugated antibody specific to IL4. Next, Avidin conjugated to HRP is added to each well and incubated. After TMB substrate addition, only those wells that contain IL4, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of IL4 in the samples is then determined by comparing the OD of the samples to the standard curve.

CHARACTERISTICS

Sample Type: Serum, plasma, tissue homogenates, cell lysates, urine, saliva, cell culture supernates and other biological fluids.

Detection Range: 15.63-1000 pg/mL
Minimum Detection Dose: 6.3 pg/mL
Intra-Assay Precision: CV<8%
Inter Assay Precision: CV<10%
Linearity with spiked analytes: ρ=0.99
Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacturing
Storage: 2-8°C

Once opened, unused wells should be put into the sealed bag with a desiccant pack and stored at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

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 Lysates - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

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

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C. Remove particulates and assay immediately or store samples in aliquot at ≤-20°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

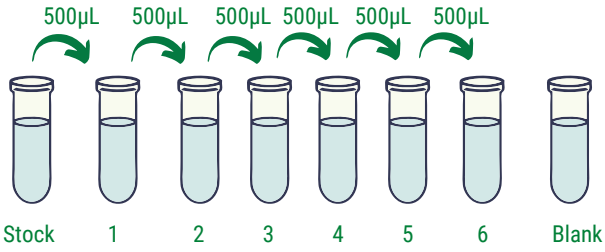
Note:

1. Samples to be used within 5 days 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

1. Bring all kit components and samples to room temperature (18-25°C) before use.

2. **Standard** -Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 1000 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.63 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



3. **Biotinylated Antibody and Streptavidin-HRP** - Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

4. **Wash Buffer** - Dilute the 25x wash buffer into 1x working concentration with double distilled water.

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100 µL each of standard working solution (read Reagent Preparation), or 100 µL of samples into the appropriate wells. Cover with the Plate sealer. Incubate for 80 minutes at 37°C.

2. Remove the liquid of each well. Aspirate the solution and wash with 200µL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

3. Add 100µL of Biotinylated Antibody working solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

4. Repeat the aspiration, wash process for total 3 times as conducted in step 2.

5. Add 100µL of Streptavidin-HRP working solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.

6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.

7. Add 90µL of TMB Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of TMB Substrate Solution.

8. Add 50 µL of Stop reagent to each well. The liquid will turn yellow by the addition of Stop reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop reagent should be the same as that of the TMB Substrate Solution.

9. Remove any drop or fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid in the plate. Then, run the microplate reader and conduct measurement at 450nm immediately.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve with the chicken IL4 concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. Use some plot software, for instance, curve expert.
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

- Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

- If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
  Making serial dilution in the wells directly is not permitted.

- 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.

- Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.

- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally leading to wrong results. Also, bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

- If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.

- The TMB solution is light sensitive. Avoid prolonged exposure to light. Avoid contact of the TMB solution with metal to prevent color development. TMB is toxic, avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.

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CUSTOMERSERVICE@LIFETECHINDIA.COM
1800-120-2434