Duck Interleukin 1 (IL-1) ELISA Kit

to determine Duck Interleukin 1(IL-1) in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT230051EAYQ

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INTENDED USE

This kit is used to determine the Duck Interleukin 1(IL-1) ELISA Kit in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *In vitro* use only.

MANUAL VERSION 1.02C

INTRODUCTION

ASSAY PRINCIPLE

The kit tests the level of Duck Interleukin 1(IL-1), based on the principle of double antibody sandwich enzyme linked immunosorbent assay (ELISA). Standards and samples are added to the wells that are pre-coated with specific antibody, then HRP-Conjugate reagent is added to form an immune complex, followed by incubation and washing, which removes unbound enzyme. Finally substrate A and B are added, which turns the solution blue and finally change into yellow in presence of an acid. The color intensity is positively correlated with the concentration of Duck Interleukin 1 (IL-1).

CHARACTERISTICS

- This Kit allows for the determination of Duck Interleukin 1(IL-1), concentrations in serum, cell culture supernates and other biological fluids. Detection range: 10pg/mL -240pg/mL
- The minimum detectable dose (MDD) of Duck Interleukin 1(IL-1), is typically less than 1.0pg/mL
- Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.
- Duck Interleukin 1(IL-1) ELISA Kit has high sensitivity and excellent specificity for detection Duck Interleukin 1(IL-1). No significant cross-reactivity or interference between Duck Interleukin 1(IL-1) and analogues was observed.

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Name	96 determinations
Microelisa Stripplate	12*8strips
Standard A	0pg/mL
Standard B	15pg/mL
Standard C	30pg/mL
Standard D	60pg/mL
Standard E	120pg/mL
Standard F	240pg/mL
Sample Diluent	6.0ml
HRP-Conjugate reagent	10.0ml
20X Wash solution	25ml
Chromogen Solution A	6.0ml
Chromogen Solution B	6.0ml
Stop Solution	6.0ml
Closure plate membrane	2
User manual	1
Sealed bags	1

[1]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.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. .37 ℃ incubator

2.Microplate reader capable of measuring absorbance at 450 nm.

3.Precision pipettes to deliver 2 ml to 1 ml volumes.

4.100 ml and 1 liter graduated cylinders.

5. Distilled water,

6. Disposable test tube

7. Absorbent paper

8. Precision pipettes and disposable tip

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

2.Blood plasma: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulation. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

3.Urine: Collect by sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

4.Cell culture supernatant: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (PH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells **through** repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed

again.

5.Tissue sample: Incise sample and weigh up. Add a certain amount of PBS (PH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add a certain amount of PBS (PH 7.4) and then homogenize the sample thoroughly by hand or homogenizer. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

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.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Micro Elisa Strip plate.

2. Add standard: Set Standard wells and sample wells. Add standard 50µl to standard wells.

3. Add Sample: ①Add Sample 10µl to test sample well, then add sample diluent 40µl to test sample well; In blank wells don't add anything.

② Add 100µl of HRP-conjugate reagent to each well (Standard wells and testing sample wells), then cover it with seal plate membrane, gently shake and mix for 60 minutes at 37 ° C incubation.

4. Preparation of washing solution: Dilute the washing concentration (20X) with distilled or deionized water for later use.

5.Washing by hand: carefully remove the sealing film, drain the liquid, fill each well with washing solution, put it aside for 1 min then drain the liquid, and repeat 5 times, pat dry. (For Automatic washing: In each well, inject

350μL wash solution, soak 1min, wash plate 5 times.)

6. Color development: first add 50μl chromogen solution A to each wells, then add 50μl chromogen solution B to each well as well. Shake gently to mix up. Incubate for 15 minutes at 37˚C, away from light for color development.

7. Stop: Add 50μl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately). If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 15 minutes after having added the stop solution.

9. According to standards' concentrations and the corresponding OD values, determine the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample.

CALCULATION

1.This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.

2.First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.

3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration. Multiply the concentration determined by the respective dilution factor as may be applicable (for example as in this case is 5)

4. Any variation in operator, pipetting and washing technique, incubation time or temperature can cause variation in result. Each user should obtain their own standard curve.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.

2.It is highly recommended to use the remaining reagents within 1 month before the deadline. For the expiration date, please refer to the label on the kit box. All components are stable before this expiration date. Do not use kit components beyond their expiration date.

3.Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

4. Use only deionized or distilled water to dilute reagents.

5. Each steps add sample, should use sampler, and often proofread the accuracy to avoid the test error. Use fresh disposable pipette tips for each transfer to avoid contamination.

6. Test should strict accordance with the instructions of the operation, the test results must be determined by the microplate reader.

7. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
8. Do not mix acid and sodium hypochlorite solutions.
9. 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.
10. All samples should be disposed of in a manner that will inactivate viruses.
11. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate Solution is easily contaminated. If bluish prior to use, do not use. Substrate B is sensitive to light and avoid prolonged exposure to light.

VALIDITY & STORAGE: Twelve months.

Storage： 2-8℃.