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Chicken Heat Shock Protein 70 (HSP-70) ELISA Kit

to determine Chicken Heat Shock Protein 70 in Serum, Blood Plasma, cell culture supernates, cell lysates, and tissue homogenates.

INSTRUCTION MANUAL

FOR ELISA KIT No: LThC4210EA

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INTENDED USE

This kit is used to assay the Chicken Heat Shock Protein 70 (HSP-70) in the sample of serum, blood plasma and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.02

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PRINCIPLE

Chicken Heat Shock Protein 70(HSP-70) ELISA Kit employs a two-site sandwich ELISA to quantitate HSP-70 in samples. An antibody specific for HSP-70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HSP-70 present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated Chicken HSP-70 detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of HSP-70 bound in the initial step. The color development is stopped and the intensity of the color is measured.

CHARACTERISTICS

- This Kit allows for the determination of HSP-70 concentrations in Chicken serum, cell culture supernatants and other biological fluids.
- Detection range: 25 ng/L - 400 ng/L.
- The minimum detectable dose (MDD) of Chicken HSP-70 is typically less than 1 ng/L.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 9%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 11%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of Chicken HSP-70 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of

the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

- Chicken Heat Shock Protein 70(HSP-70) ELISA Kit has high sensitivity and excellent specificity for detection of Chicken HSP-70. No significant cross-reactivity or interference between Chicken HSP-70 and analogues was observed.

MATERIALS SUPPLIED IN THIS KIT

components	48T	96T	Storage conditions
Chicken HSP-70 microplate	48 wells	96 wells	2-8 °C[1]
Chicken HSP-70 standard	0.5 mL	0.5 mL	2-8 °C
HRP-Conjugated Chicken HSP-70 detection antibody	3 mL	6 mL	2-8 °C
Standard diluent	1.5 mL	1.5 mL	2-8 °C
Sample diluent	3 mL	6 mL	2-8 °C
Chromogen solution A	3 mL	6 mL	2-8 °C
Chromogen solution B	3 mL	6 mL	2-8 °C
Stop solution	3 mL	6 mL	2-8 °C
Wash buffer	20 mL (20×)	20 mL (30×)	2-8 °C
Plate covers	1	2	RT
Booklet	1	1	RT

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.

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MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips 4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

SAMPLE PREPARATION

1.Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).

2.After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

3.Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4.Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5.Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.

6.Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and 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 by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

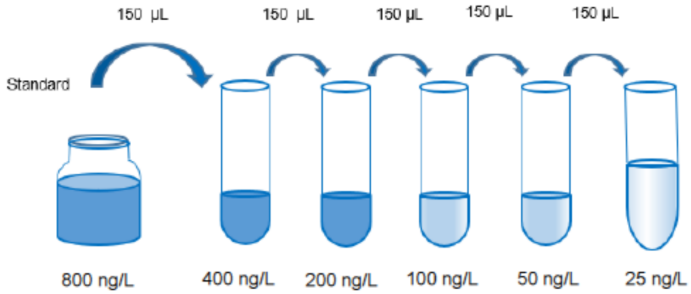
7.Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8℃. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) 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 were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:30.

Standard - Pipette 150 µL of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard.



ASSAY PROCEDURE

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

2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 µL to standard well.

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3. Add Sample: Add sample diluent 40 µL to testing sample well. Then add sample 10 µL to testing sample well, in Blank well do not add anything.

4. Cover with a plate cover and incubate for 45 minutes at 37 °C.

5. Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 µL) using a squirt bottle, manifold dispenser or auto-washer. 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.

6. Add HRP-Conjugated detection antibody 50µL to each well, except blank well.

7. Cover with plate cover. Incubate for 30 minutes at 37 °C.

8. Repeat the aspiration/wash process for five times as in step 5.

9. Add chromogen solution A 50 µL and chromogen solution B 50 µL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10. Add 50 µL 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

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 mean absorbance for each standard on the x-axis against the concentration 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 Chicken HSP-70 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 (e.g. 5 in this case).

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- 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.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
- Stop Solution should be added to the plate in the same order as the Chromogen

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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 Chromogen solution.

- 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.
- All samples should be disposed of in a manner that will inactivate viruses. 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.

PACKAGE SIZE： 96Tests.

VALIDITY & STORAGE: Twelve months from date of purchase.

Avoid repeated freeze thaw cycles.