Science for Society

Human Cholera antibody ELISA Kit

to determine Human Cholera antibody in Serum, Blood Plasma, Saliva,

Urine, And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT06826ETKBA

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INTENDED USE

This kit is used to determine the Human Cholera antibody in the sample of serum, blood plasma, saliva, urine, and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.02

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PRINCIPLE

Human Cholera antibody ELISA Kit quantitate Human Cholera antibody in samples. A purified antigen has been pre-coated onto a microplate. Standards or test samples are added to the wells, incubated and then washed. An antigen-HRP conjugated is then added and incubated. The plate is washed and the chromogen solution is then added which is catalysed by HRP, generating a blue coloration after incubation. A stop solution is added which generates conversion to yellow color read at 450 nm which is proportional to the amount of analyte bound.

MATERIALS SUPPLIED IN THIS KIT

Human Cholera antibody microplate: 96 well polystyrene microplates (8 strips of 12 wells) coated with the antigen.

Human Cholera antibody standard: Human Cholera antibody in a buffered protein base with preservatives, liquid.

Standard diluent: Diluent solution for reconstituted standard.

Sample diluent: Diluent solution for reconstituted samples.

HRP-Conjugate antigen: HRP Conjugate antigen, liquid.

Chromogen solution A: liquid.

Chromogen solution B: liquid.

Stop solution: liquid.

Wash buffer: 30× liquid.

Plate covers.

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

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay): Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) <9%.

Inter-assay Precision (Precision between assays): 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%.

Recovery: The recovery of Human Cholera antibody spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.

The recovery ranged from 98% to 116% with an overall mean recovery of 106%

Sensitivity: The minimum detectable dose (MDD) of Human Cholera antibody is typically less than 1.0 ng/L. The MDD was determined by adding two standard deviations to the mean O.D. value twenty zero standard replicates and calculating the corresponding concentration.

Specificity: Human Cholera antibody ELISA Kit can be used to measure Human Cholera antibody in samples. Human Cholera antibody ELISA Kit has high sensitivity and excellent specificity for detection of Human Cholera antibody. No significant cross-reactivity or interference between Human Cholera antibody and analogues was observed.

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 °C. 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.

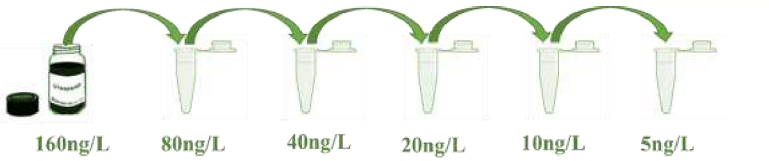
6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. 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 °C. 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.

ASSAY PROCEDURE

1. Dilution of standard solution: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

80 ng/L	Standard No.5	150µl Original Standard + 150µl Standard diluents
40 ng/L	Standard No.4	150µl Standard No.5 + 150µl Standard diluents
20 ng/L	Standard No.3	150µl Standard No.4 + 150µl Standard diluent
10 ng/L	Standard No.2	150µl Standard No.3 + 150µl Standard diluent
5 ng/L	Standard No.1	150µl Standard No.2 + 150µl Standard diluent



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

3. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50µl to standard well.

4. Add Sample: Add sample diluent 40 µl to testing sample well. Then add sample 10µl to testing sample well; for Blank well don't add anything.

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

6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle, manifold dispenser or autowasher. 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.

7. Add 50 µl HRP-Conjugate to each well, except blank well.

8. Cover with a new adhesive strip. Incubate for 30 minutes at 37 °C.

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

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

11. Add 50µl 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.

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

PROTOCOL SUMMARY

Prepare reagents, samples and standards.

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Add prepared samples and standards in respective wells together and incubate for 45 minutes at 37 °C.

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Wash the plate five times. Add HRP-Conjugate and incubate for 30 minutes at 37 °C.

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Wash the plate five times. Add Chromogen solution A and B. Incubate for 15 minutes at 37 °C for color development and add stop solution.

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Read the OD value within 15 minutes.

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Plot and Calculate.

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 y-axis against the concentration for each standard on the x-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 Cholera antibody 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.

ASSAY RANGE : 5 ng/L - 80 ng/L

PACKAGE SIZE : 96Tests.

SENSITIVITY : <1.0 ng/L.

Linearity: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human Cholera antibody 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.

VALIDITY & STORAGE: Six months (at 2-8°C).