Human Typhoid IgG ELISA Kit

to determine Human Typhoid IgG in Serum, Blood Plasma, Saliva, Urine,

And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT37826ETKBA

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INTENDED USE

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

MANUAL VERSION 1.02

BACKGROUND

Typhoid fever is a type of enteric fever along with paratyphoid fever. Symptoms may vary from mild to severe and usually begin six to thirty days after exposure. Often there is a gradual onset of a high fever over several days. The cause is the bacterium *Salmonella typhi*, also known as Salmonella enterica serotype Typhi, growing in the intestines and blood. Typhoid is spread by eating or drinking food or water contaminated with the feces of an infected person. Risk factors include poor sanitation and poor hygiene.

PRINCIPLE

Human Typhoid IgG ELISA Kit quantitate Human Typhoid IgG in samples. A antigen has been pre-coated onto a microplate. A purified antigen has been pre-coated onto a microplate. Standards or test samples are added to the wells, incubated and then washed. An HRP conjugate is then added and incubated. The plate is washed once more and the chromogen solution is then added which HRP catalyzes, 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.

ASSAY RESTRICTIONS

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

• It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.

• If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

• Any variation in standard diluent, operator, pipetting technique, washing

technique, incubation time or temperature, and kit age can cause variation in binding.

• 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.

MATERIALS SUPPLIED IN THIS KIT

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

Human Typhoid IgG standard: Human Typhoid IgG in a buffered protein base with preservatives, liquid.

Standard diluent: Diluent solution for reconstituted standard.

Sample diluent: Diluent solution for reconstituted samples.

HRP-Conjugate: HRP Conjugate, liquid.

Chromogen solution A: liquid.

Chromogen solution B: liquid.

Stop solution: liquid.

Wash buffer: 30X 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

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C for 6 months. Immediately after use

remaining reagents should be returned to cold storage at 4˚C. Recommended storage instruction for opened/reconstituted kit components are listed below.

Kit components	Storage conditions for opened components
Human Typhoid IgG microplate	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.
Human Typhoid IgG standard	Stored at 2-8°C.
HRP-conjugate, Standard Diluent, Sample Diluent, Chromogen solution A, Chromogen solution B, Stop Solution, Wash buffer	Stored at 2-8 °C.

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. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. 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.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. 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 Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

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

10. 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.

11. Chromogen Solution is easily contaminated. If bluish prior to use, do not use.

12. Chromogen B contain 20% acetone, keep this reagent away from sources of heat or flame.

13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

TECHNICAL HINTS

• 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 should remain colorless until added to the plate. Keep

Chromogen solution protected from light. Chromogen solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Chromogen 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.

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 Typhoid IgG 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 Typhoid IgG is typically less than 1.0 IU/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 Typhoid IgG ELISA Kit can be used to measure Human

Typhoid IgG in samples.

Human Typhoid IgG ELISA Kit has high sensitivity and excellent specificity for detection of Human Typhoid IgG. No significant cross-reactivity or interference between Human Typhoid IgG and analogues was observed.

SAMPLE COLLECTION & STORAGE

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

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should 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 which will be homogenized in PBS (the volume depends on the weight of the tissue. 9ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernatants 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 for later use. Avoid repeated freeze/thaw cycles.

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 approximately 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 1000×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. Samples should be centrifugated adequately and no hemolysis or granule was 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.

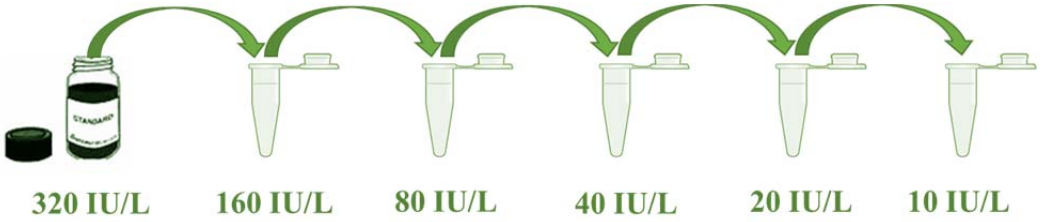
Wash buffer - Dilute with Distilled or deionized water 1:30.

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. Standard Diluent serves as the zero standard.

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:

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

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

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

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.

Read the OD value within 15 minutes.

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 Typhoid IgG 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：10 IU/L - 160 IU/L.

PACKAGE SIZE：96Tests.

SENSITIVITY：< 1.0 IU/L.

Linearity: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human Typhoid IgG 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℃, unopened).