

Product Information

***Salmonella typhi* IgG ELISA kit**

Catalog Number: EA100959

Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The *Salmonella* IgG ELISA Kit is intended for the detection of IgG antibody to *Salmonella* in human serum or plasma.

Background

Salmonella typhi is the causative agent of typhoid fever a contagious infection of the intestines that affects the whole body. In developing countries, typhoid often occurs in epidemics. Most people in the United States get typhoid as a result of visiting another country where the food or water supply has been contaminated. Symptoms usually start 1 to 3 weeks after exposure to the bacteria. Symptoms include: high fever, headache, sore throat, vomiting, diarrhea, skin rash and weakness. The symptoms may take 2 weeks or more to go away. Typhoid is spread when a person drinks or eats food and water contaminated by human waste (stool or urine) containing *Salmonella typhi* bacteria. A person who no longer has symptoms may still transmit the bacteria as a carrier. Testing for immunoglobulin G (IgG), IgA, and IgM anti-lipopolysaccharide (LPS) of *Salmonella typhi* antibodies by enzyme-linked immunosorbent assay (ELISA) showed that the levels of all three classes of immunoglobulin anti-LPS of *S. typhi* were higher in typhoid patients than in healthy or febrile nontyphoidal groups. The ELISA assay was much more sensitive and specific than any combination of the Widal test, and hence it could be a useful tool for the serologic diagnosis of typhoidal fever with a single blood sample.

Principle of the Test

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with <i>Salmonella typhi</i> antigen	12x8x1
2. Sample Diluent: 2 bottles (ready to use)	2 x 25 ml
3. Calibrator: 1 Vial (ready to use)	1ml

4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12 ml
7. TMB Substrate: 1 bottle (ready to use)	12 ml
8. Stop Solution: 1 bottle (ready to use)	12 ml
9. Wash concentrate 20X: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
 - The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
 - It is recommended that standards, control and serum samples be run in duplicate
 - Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities
1. Place the desired number of coated strips into the holder.
 2. Negative control, positive control, and calibrator are ready to use. Prepare 1:101 dilution of test samples, by adding 5 µl of the sample to 0.5 mL of sample diluent. Mix well.
 3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
 4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
 5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
 6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

7. Dispense 100 μ l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 μ l of stop solution.
8. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Limitations of the Test

1. Lipemic or hemolyzed samples may cause erroneous results.

References

1. Quiroga T; Goycoolea M; Tagle R; Gonzalez F; Rodriguez L; Villarroel L. Diagnosis of typhoid fever by two serologic methods. Enzyme-linked immunosorbent assay of antilipopolsaccharide of Salmonella typhi antibodies and Widal test. *Diagn Microbiol Infect Dis* 1992; 15(8):651-6.
2. Jesudason MV; Sridharan G; Arulselvan R; Babu PG; John TJ. Diagnosis of typhoid fever by the detection of anti- LPS & anti-flagellin antibodies by ELISA. *Indian J Med Res* 1998;107:204-7.
3. Mekara Y; Maneekarn N; Vithayasai V; Makonkawkeyoon S. Determination of antibody from typhoid patients against lipopolysaccharide and protein antigens of Salmonella typhi. *Asian Pac J Allergy Immunol* 1990; 8(2):95- 101.
4. Sippel JE; Hanafy HM; Diab AS; Prato C; Arroyo R. Serodiagnosis of typhoid fever in pediatric patients by anti-LPS ELISA. *Trans R Soc Trop Med Hyg* 1987; 81(6):1022-6.
5. Vitale G; Librizzi R; Mocciaro C; Friscia I; Blandino E; Usticano V; Mansueto S; Di Fiore M; Reina G; Gambino G. An ELISA method in the diagnosis of typhoid fever. *J Clin Lab Immunol* 1990; 31(4):195-9.