3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

***Salmonella typhi* IgM ELISA**

Catalog Number **SE120112**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

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 antilipopolysaccharide (LPS) of *Salmonella typhi* antibodies by Enzyme-Linked Immunosorbent Assay (ELISA) showed 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.

The *Salmonella* IgM ELISA Kit is intended for the detection of IgM antibody to *Salmonella* in human serum or plasma. Diluted serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgM 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 TMB Substrate is added. The plate is incubated to allow the oxidation of the Substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

Components

Materials Provided	96 Tests
Microwells coated with *Salmonella typhi* antigen	12 x 8 x 1
Sample Diluent: 2 bottle (ready to use)	25 mL
Calibrator: 1 vial (ready to use)	1 mL
Positive Control: 1 vial (ready to use)	1 mL
Negative Control: 1 vial (ready to use)	1 mL
Enzyme conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
Wash concentrate 20x: 1 bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample 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.

20x Wash Buffer Concentrate

Prepare 1x Wash Buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator is >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 101-fold 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 of 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 (18–26 °C).
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
5. Dispense 100 µL of Enzyme Conjugate to each well and incubate for 20 minutes at room temperature (18–26 °C).
6. Remove Enzyme Conjugate from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
7. Dispense 100 µL of TMB Substrate and incubate for 10 minutes at room temperature (18–26 °C).
8. Add 100 µL of Stop Solution.
9. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.

Results
Calculations
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD × 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.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Notes: To enhance sensitivity and specificity of this IgM test, the provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences.

Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.

In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.

Lipemic or hemolyzed samples may cause erroneous results.

Interpretation
The following is intended as a guide to interpretation of *Salmonella typhi* IgM antibody index (Ab Index) test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

<0.9 – No detectable antibody to *S. typhi* IgM by ELISA
0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1 – Detectable antibody to *S. typhi* IgM by ELISA

References

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2. Jesudason, M.V. et al., 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. et al., 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, J.E. et al., 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. et al., An *ELISA* method in the diagnosis of typhoid fever. J. Clin. Lab. Immunol., 1990; 31(4):195-9.

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