REFERENCES

Salmonella Typhi IgG ELISA

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

SUMMARY AND EXPLANATION
Salmonella typhi is the causative agent of typhoid fever, a contagious infection of the intestines that affects the whole body. In developing countries, typhoid occurs frequently 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 antipolyosaccharide (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 non-typhoidal 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.

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 Tests</th>
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<tbody>
<tr>
<td>1. Microtiter coated with Salmonella typhi antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. Sample Diluent: 2 bottle (ready to use)</td>
<td>25 ml</td>
</tr>
<tr>
<td>3. Calibrator: 1 Vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>4. Positive Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>5. Negative Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>9. Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

MATERIALS 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
6. Graph paper

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. -
1. Place the ELISA reader plate in the well plate. The wells are numbered clockwise from the top row to the bottom row.
2. Add 100 μL of the sample extract or standard to each well. Ensure the samples are distributed evenly.
3. Add 100 μL of the conjugate to each well. Incubate for 1 hour at room temperature.
4. Remove the excess conjugate from the wells by washing with 200 μL of wash buffer. Repeat this step 3 times.
5. Add 100 μL of the substrate solution to each well. Incubate for 10 minutes at room temperature.
6. Add 50 μL of the stop solution to each well. Mix well and measure the absorbance at 450 nm.
7. Calculate the absorbance and subtract the blank values. The concentration of the sample can be determined by comparing it to the standard curve.

**WARNING AND PROCEDURES**

1. Use caution when handling the reagents and samples. Wear appropriate personal protective equipment.
2. Keep reagents and samples out of the reach of children.
3. Dispose of all reagents and samples according to institutional guidelines for waste management.
4. Proceed with the assay only after the reagents have reached room temperature.