Salmonella ELISA Test Kit Manual
Catalog #: 1039
Reference #: 1039-03

This kit is manufactured to the international quality standard ISO 9001:2008.
ISO C#: SARA-2009-CA-0114-01-B

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*MaxSignal® Salmonella ELISA Test Kit is intended for laboratory use only, unless otherwise indicated. This product is NOT for clinical diagnostic use. MaxSignal is a registered trademark of Bioo Scientific Corporation (BIOO).*
**General Information**

**Product Description**

MaxSignal® Salmonella ELISA Test Kit is designed for routine screening for Salmonella from cultured food and feed samples. Salmonella is a genus of gram negative bacteria belonging to the Enterobacteriaceae family. As a major contributor to foodborne illness, Salmonella is founded in poultry, meat and eggs. When Salmonella is consumed it may result in the disease known as Salmonellosis. The detection of Salmonella in food samples is often laborious and time-consuming. Several days are required to culture the bacteria from a sample followed by a final isolation and identification by biochemical and serological techniques. The MaxSignal® Salmonella ELISA Test Kit provides a simple, rapid, sensitive and cost-effective enzyme immunoassay (ELISA) screening method, which enables government agencies, seafood processors, as well as quality assurance organizations, to detect positive and negative Salmonella samples to as low as 10^5 cells/mL level.

**Procedure Overview**

The method is based on a colorimetric ELISA assay. The monoclonal antibody against Salmonella has been coated in the plate wells. During the analysis, the sample is added. If Salmonella is present in the sample, it will bind with the antibody. A suitable broth is added to the well for rapid growth. The secondary antibody, tagged with a peroxidase enzyme, targets the Salmonella that is complexed to the primary antibody coated on the plate wells. The resulting color intensity, after addition of substrate, is related to the amount of Salmonella in the sample.

**Kit Contents, Storage and Shelf Life**

MaxSignal® Salmonella ELISA Test Kit has the capacity for 96 determinations or testing of 46 samples in duplicate (assuming 4 wells for positive and negative controls). Most of the kit components should be stored at 2-8°C except for the Salmonella controls and Ab#2 stock which should be stored at -20°C. Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. The shelf life is 12 months when the kit is properly stored.

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella antibody-coated Plate</td>
<td>1 x 96-well plate (8 wells x 12 strips)</td>
<td>2-8°C</td>
</tr>
<tr>
<td>1X Salmonella Negative Control</td>
<td>1.0 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>1X Salmonella Positive Control</td>
<td>1.0 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>HRP-Conjugated Antibody #2 (powder)</td>
<td>3 tubes</td>
<td>-20°C</td>
</tr>
<tr>
<td>Antibody #2 Diluent **</td>
<td>20 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>20X Wash Solution **</td>
<td>28 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Buffer **</td>
<td>14 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>TMB Substrate **</td>
<td>12 mL</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>

** Components with the same BIOO part No’s within their expiration dates are interchangeable among BIOO kits.
**Sensitivity (Detection Limit)**

The kit can detect 10⁵ cells/mL.

**Specificity (Cross-Reactivity)**

The kit can broadly detect *Salmonella* serotypes of group A, B, C, D, E and other *Salmonella* antigen classes. The intensity of reaction may vary with the strain of *Salmonella* tested.

**Required Materials Not Provided With the Kit**

- Microtiter plate reader (450 nm)
- Incubator
- Vortex mixer, (e.g. Geneie Vortex mixer from VWR)
- 10, 20, 100 and 1000 µL pipettes
- Multi-channel pipette: 50-300 µL (Optional)
- Modified GN Broth: GN broth containing 10µg/ml Novobiocin. GN broth can be purchased or prepare in-house per the following protocol:

  **Gram Negative Broth (GN Broth or Hajna GN Broth, pH 7.0 ± 0.2)**
  
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium desoxycholate</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>4.0 g</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.0 L</td>
</tr>
</tbody>
</table>

Dissolve ingredients in water. Autoclave at 121°C for 15 min. Avoid overheating.

- Autoclave or boiling water bath (100°C)
- Culture bottles (500ml)
- Microbiological media for enrichment of *Salmonella*
- Microbiological media for the confirmation of *Salmonella*
- Distilled water
Warnings and Precautions

BIOO strongly recommends that you read the following warnings and precautions to ensure your full awareness of ELISA techniques and other details you should pay close attention to when running the assays. More information can also be found in Troubleshooting section. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport@biooscientific.com.

- The samples contain *Salmonella*. Handle with particular care.
- Good microbiological practices (i.e. aseptic technique, safety precautions) should be employed when carrying out sample preparation and enrichment procedures.
- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or lots except for components with the same part No’s within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC. Make sure that the antibody#2 and diluent are mixed in correct volumes.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
- Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
- Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
- Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20 – 25°C / 68 – 77°F) while in the packaging.

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SAMPLE PREPARATION

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temp. (20 – 25°C / 68 – 77°F) or in a refrigerator before use.

**Food sample**

Add 25g of sample to 225 mL of pre-enrichment broth (e.g. buffered peptone water, BPW). The sample may require blending. Incubate for 18-24 hours at 37°C. The broth may be stored refrigerated for up to 48 hours.

**SALMONELLA ELISA TEST KIT PROTOCOL**

**Reagent Preparation**

**IMPORTANT:** All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure you read “Warnings and Precautions” section on page 3. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. **Preparation of 1X Wash Solution**
   Mix 1 volume of 20X Wash Solution with 19 volumes of distilled water.

2. **Preparation of 1X HRP-Conjugated Antibody #2**
   Based on the needed amount, take out one or more HRP-Conjugated Antibody #2 tubes (powder), add 2.5 mL of Antibody #2 Diluent to each tube, invert the tube up and down for 10 times, leave the tube at room temperature for at least 15 min, invert the tube up and down for another 10 times. The antibody#2 solution is ready to use. The reconstituted antibody#2 is stable for 1 week at -20°C freezer.

**ELISA Testing Protocol**

Label the individual strips that will be used and aliquot reagents as the following example:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume per Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1X Negative Control</td>
<td>50 µL</td>
</tr>
<tr>
<td>1X Positive Control</td>
<td>50 µL</td>
</tr>
<tr>
<td>1X HRP-Conjugated Antibody #2</td>
<td>50 µL</td>
</tr>
<tr>
<td>1X Wash Solution</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>Stop Buffer</td>
<td>100 µL</td>
</tr>
<tr>
<td>Substrate</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

1. Take out the needed plate strips, add 50 µL of Negative and Positive Control in duplicate into different wells.
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Incubate the plate for 30 minutes at 37°C.

4. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash the plate 3 times with 300 μL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).

5. Add 250 μL Modified GN Broth to each well. Cover the wells and incubate for 4 hours at 37 °C. Transfer the well broth to another container if needed for future validation (optional). Wash the plate 3 times with 300 μL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels.

6. Add 50 μL of 1X HRP-Conjugated Antibody #2 to each well. Incubate the plate for 30 minutes 37°C (Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).

7. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash the plate 3 times with 300 μL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).

8. Add 100 μL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).

9. After incubating for 15 minutes at room temperature (20 – 25 °C / 68 – 77 °F), add 100 μL of Stop Buffer to stop the enzyme reaction.

10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

**Interpretation of the Results**

**Visual Interpretation of the Results**

Visual assessment must be done prior to addition of stopping solution.

- **Positive Control:** Blue
- **Negative Control:** Clear or very pale blue

A sample is considered positive when the following criteria are met:

1. The negative control is clear or very pale blue in color and the positive sample is significantly deeper in color than this.
2. The positive sample should always be significantly deeper in color than the negative control, however it need not be as blue as the positive control.

A sample is considered negative when the following criteria are met:

1. The negative control is clear or very pale blue in color.
2. The sample is less than or equal to the color of the negative control.
Interpret results and record as positive or negative on the record sheet provided. Return reagents to 2-8°C. Negative/Positive samples can only be interpreted if the expected results are produced with the controls.

**OD Method**

Measure the plate absorbance using a microplate reader at A450nm and record absorbance values on the record sheet provided.

- **Negative Control:** Readings at A450nm should give an OD of less than 0.4. If not this indicates insufficient washing of the microplate.
- **Positive Control:** Readings at A450nm should give an OD greater than 1.0.

If controls are not within the limits noted above, the test must be repeated.

- **Negative samples:** Samples that give A450nm < 0.6
- **Positive samples:** Samples that give A450nm ≥ 0.6

Negative/positive samples can only be interpreted if the expected results are produced with the controls.

If confirmation of *MaxSignal® Salmonella ELISA Test* positive samples is required, we would recommend one of the following confirmation procedures.

1. Transfer 1ml of each of the suspect pre-enriched sample broths to a fresh 10 mL aliquot of mGN broth and incubate at 42°C for 18-24 hours. At the same time, streak each of the mGN broths to at least two salmonella selective agar media and incubate for 18-24 hours at 35°C as instructed by the manufacturer. Normally Xylose Lysine Desoxycholate agar (XLD), Hektoen-Enteric agar and Bismuth Sulphite agar are used.

2. Examine selective agar media for suspicious colonies and, if present, confirm these using biochemical and serological testing.
## TROUBLESHOOTING

### No Color Development or No Signals with Standards

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents were used in the wrong order or a step was skipped.</td>
<td>Follow the protocol carefully and repeat the assay.</td>
</tr>
<tr>
<td>Wrong antibodies were used, or antibody #2 was prepared incorrectly or has deteriorated.</td>
<td>Make sure that the antibodies used are the ones that came with the kit. All antibodies are kit- and lot-specific. Make sure that the antibody #2 and diluent are mixed in correct volumes.</td>
</tr>
<tr>
<td>TMB substrate has deteriorated.</td>
<td>Use a new set of BIOO TMB substrate.</td>
</tr>
</tbody>
</table>

### Low Optical Density (OD) Readings

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents were expired or mixed with a different lot number.</td>
<td>Verify the expiration dates and lot numbers.</td>
</tr>
<tr>
<td>Wash solution was prepared incorrectly.</td>
<td>Use the wash solution in the kit and make sure that it is prepared correctly.</td>
</tr>
<tr>
<td>Too many wash cycles were used.</td>
<td>Make sure to use the number of washes per the protocol instruction.</td>
</tr>
<tr>
<td>Incubation times were too short.</td>
<td>Time each plate separately to ensure accurate incubation times, follow protocol.</td>
</tr>
<tr>
<td>Lab temperature was too low.</td>
<td>Maintain the lab room temperature within 20°–25°C (68°–77°F). Do not run assays under air conditioning vents or near cold windows.</td>
</tr>
<tr>
<td>Reagents and plates were too cold.</td>
<td>Make sure plates and reagents are brought up to room temperature. Keep the kit components out of the kit box for at least 1 hour before starting the assay.</td>
</tr>
<tr>
<td>Reader was at wrong wavelength, or reader was malfunctioning.</td>
<td>Make sure the wavelength is 450 nm for the assay and read the plate again.</td>
</tr>
<tr>
<td>Excessive kit stress has occurred.</td>
<td>Check records to see how many times the kit has cycled from the refrigerator.</td>
</tr>
<tr>
<td>Assay plates were compromised</td>
<td>Always refrigerate plates in sealed bags with a desiccant to maintain stability.</td>
</tr>
</tbody>
</table>

### High Background or High Optical Density (OD) Readings

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor quality water was used in wash solution.</td>
<td>If water quality is questionable, try substituting an alternate distilled water source to prepare the wash solution.</td>
</tr>
<tr>
<td>Substrate solution has deteriorated.</td>
<td>Make sure the substrate is colorless prior to addition to the plate.</td>
</tr>
<tr>
<td>There was insufficient washing or poor washer performance.</td>
<td>Use the number of washes per the protocol instruction. Make sure that at least 250 µL of wash solution is dispensed per well per wash. Verify the performance of the washer system; have the system repaired if any ports drip, dispense or aspirate poorly.</td>
</tr>
<tr>
<td>Reader was malfunctioning or not blanked properly. This is a high possibility if the OD readings were high and the color was light.</td>
<td>Verify the reader’s performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and reblank.</td>
</tr>
<tr>
<td>Lab temperature was too high.</td>
<td>Maintain the room temperature within 20°–25°C (68°–77°F). Avoid running assays near heat sources or in direct sunlight.</td>
</tr>
<tr>
<td>Reagents were intermixed, contaminated or prepared incorrectly.</td>
<td>Ensure that the correct reagents were used, that working solutions were prepared correctly and that contamination has not occurred.</td>
</tr>
</tbody>
</table>
### High Intra-Plate Variance

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inconsistent time was taken when adding standards, reagents or samples to the assay plate.</td>
<td>Make sure all materials are set up and ready to use. Use a multichannel pipette to add reagents to multiple wells whenever possible. Do not interrupt while adding standards, reagents and samples.</td>
</tr>
<tr>
<td>Multichannel pipette was not functioning properly.</td>
<td>Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.</td>
</tr>
<tr>
<td>There was inconsistent washing or washer system malfunctioning.</td>
<td>Check performance of the wash system. Have the system repaired if any ports drip or dispense/aspirate poorly.</td>
</tr>
</tbody>
</table>

### High Inter-Plate Variance

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inconsistent incubation times occurred from plate to plate.</td>
<td>Time each plate separately to ensure consistent incubation times.</td>
</tr>
<tr>
<td>Inconsistent washing occurred from plate to plate.</td>
<td>Make sure to use the number of washes per the protocol instruction. Verify performance of the wash system and have the system repaired if any ports drip or dispense/aspirate poorly.</td>
</tr>
<tr>
<td>Pipette was working improperly.</td>
<td>Check the pipette calibration. Verify that pipette tips are on tight before use and that all channels draw and dispense equal volumes.</td>
</tr>
<tr>
<td>Kit plates, reagents, standards and samples were at different temperatures.</td>
<td>Make sure to allow sufficient time for kit plates, reagents, standards and samples come to room temperature (20 – 25°C / 68 – 77°F). Larger volumes will require longer equilibration time. If using a water bath to hasten equilibration, make sure it is maintained at room temperature; do not use a warm water bath to warm reagents, samples and kit standards.</td>
</tr>
<tr>
<td>Reagents used were intermixed from different kit lots, or the kits were of different expiration dates.</td>
<td>Carefully label each reagent to make sure the reagents are not intermixed. Kits with different expiration dates might generate different range of OD readings, however, the relative absorbance values may very well be comparable. In general, a value of less than 0.6 in positive control reading may indicate certain degrees of deterioration of reagents.</td>
</tr>
</tbody>
</table>

### One or More of the Standard Curve Points Are Out of Range

<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Recommended Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards were added in wrong order or recorded in wrong position.</td>
<td>Follow the protocol and re-run the assay. Make sure the standards are applied and recorded correctly.</td>
</tr>
<tr>
<td>Standards were contaminated or intermixed with other standards.</td>
<td>Use a new set of standards. Add standards to plate only in the order from low concentration to high concentration.</td>
</tr>
<tr>
<td>There was inconsistent washing or washer system malfunctioning.</td>
<td>Perform washing consistently. Check performance of the wash system. Have the system repaired if any ports drip or dispense/aspirate poorly.</td>
</tr>
<tr>
<td>Inconsistent time was taken to add standards and reagents to plate.</td>
<td>Make sure all materials are set up and ready to use. Add standards to plate only in the order from low concentration to high concentration at undisrupted pace. Use a multichannel pipette to add reagents to multiple wells simultaneously.</td>
</tr>
<tr>
<td>Multichannel pipette was not functioning properly.</td>
<td>Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.</td>
</tr>
</tbody>
</table>