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Food safety excellence

Solus One *Salmonella*

Immunoassay
Based Test System
For the Detection
of *Salmonella*
in Selected Foods

www.solusscientific.com

Product Number: SAL1-0096

Solus One *Salmonella* is for the next day detection of *Salmonella* in foods (Ready-to-eat (RTE) / Ready-to-reheat (RTRH) (excluding smoked products), Heat processed milk and dairy products, Egg products)

**1. INTRODUCTION**

Solus One *Salmonella* provides a negative or a presumptive positive result from a single enrichment step within 24 hours.

2. INTENDED USE

The test method requires laboratory facilities plus trained personnel. Basic training is recommended to first time users and can be provided by Solus Scientific. Using the method includes compliance with Good Laboratory Practices (refer to EN ISO 7218).

3. REAGENTS PROVIDED

Most kit components are supplied stabilised and ready to use at working concentration. Only the Washing Buffer Activator and Washing Buffer reagent require dilution.

The activator must be first dissolved in 240ml of deionised (DI) water followed by the addition of the 10ml concentrated Washing Buffer reagent to this solution.

Each kit contains sufficient material for 1 x 93 determinations, plus controls.

The kit expiry date is displayed on each product label.

- 1 x 96 well microplate (in breakable strip format). Wells are coated with antibodies against *Salmonella* spp.
- Negative Control (Green label). 3ml in working dilution. Contains diluent with preservative.
- Positive Control (Red label). 3ml in working dilution. Contains heat-killed *Salmonella* in diluent with preservative.
- Conjugate (Orange label). 11ml in working dilution. Contains horseradish peroxidase-antibody conjugate in diluent with preservative.
- Substrate (Blue label). 11ml in working dilution. Contains 3,3',5,5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution should be colourless.
- Stop Solution (Silver label). 11ml in working dilution. Contains 0.2M sulphuric acid.
- Washing Buffer Concentrate (25x). 6 x 10ml.
- Washing Buffer Activator. 6 x 1 Sachet

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C
- Deionised or distilled water
- Buffered Peptone Water (BPW) according to ISO 6579
- Solus One *Salmonella* Supplement
- 70% v:v Ethanol

- Measuring cylinder for 250ml
- Filter bags
- Frit filters
- Homogeniser
- 3ml transfer pipettes (sterile)
- Tube for sample boiling (e.g. 5ml Poly propylene rimless tubes 12x75mm)
- Vortex mixer
- Timer
- Incubator or water bath at $41.5\pm 1^{\circ}\text{C}$
- Heating block or water bath (capable of heating to $85\text{-}100^{\circ}\text{C}$)
- Pipettes and tips
- Dynex DS2 or Microplate washer and microplate reader with 450nm filter
- Autoclave for decontamination of samples

5. REAGENT PREPARATION

5.1. Washing Buffer: Prepare the following in a clean 250ml vessel –

5.1.1 Add the contents of a Washing Buffer Activator Sachet to 240ml DI water and mix until the activator has fully dissolved.

5.1.2 Add 10ml (1 bottle) of the concentrated washing buffer reagent to the vessel containing 240ml of the dissolved activator solution.

5.2. Culture Broth (growth medium): Prepare Buffered Peptone Water (BPW) ISO6579 following manufacturer's instructions. Allow to cool to room temperature before use in testing.

5.3. Prepare the Solus One *Salmonella* Supplement according to the manufacturer's instructions. Allow the contents to dissolve into solution, this typically takes around 30 minutes at room temperature. Add 4.44ml of supplement per 1L (1ml per 225ml) of BPW.

6. SAMPLE PREPARATION AND ENRICHMENT- standard method

Homogenise 25g of the sample in 225ml of supplemented BPW, and incubate for 21 ± 1 hour at $41.5\pm 1^{\circ}\text{C}$.

In the context of NF VALIDATION, test portions weighing more than 25g have not been tested. Refer to EN ISO 6579 for the specific preparations of the mother suspension for some foods.

Some enriched sample types can contain particulates that make pipetting difficult. Use of a filter bag for enrichment can contain these particulates and eliminate pipetting issues.

Ensure that the bench processing time of supplemented BPW inoculated samples is kept to a minimum and transferred to the 41.5°C incubator as soon as possible. This is important to avoid extensive growth of competing organisms.

Post enrichment

When the incubation period in supplemented BPW is completed, carefully remove a 1ml aliquot, to a glass or polypropylene tube.

Heat the aliquot at 85-100°C for 15-20 minutes in the tube. After heating allow the sample to cool to room temperature. This may be accelerated by placing the tubes in cold water for 5 minutes.

Some sample types can contain particulates that fail to settle after the heating step. We recommend separating out these particulates to prevent pipetting issues on the Dynex instrument and minimise plate washing problems. This can be achieved with an inert filter or frit (FRI001) that is pushed directly into the tube, to force any particulates to the bottom of the tube thus allowing pipetting of a relatively clear sample from above the level of the frit.

Some sample types can coagulate during the heat inactivation step, which can cause difficulties in sample pipetting. Examples of such sample types are egg-based products and caseinates. These samples may require the use of frits or in some cases manual addition to the immunoassay.

If using the Dynex instrumentation care must be taken to avoid bubbles or sample films and it is essential to check that the system has successfully pipetted a sample before proceeding.

Keep the non-heat treated samples for verification until immunoassay results are obtained. These samples can be kept at 41.5±1°C if the immunoassay test is to be carried out within 2 hours or at 2-8°C for up to 72 hours prior to the immunoassay test.

7. IMMUNOASSAY PROCEDURE

7.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature. Determine the number of wells required for the test. Take the required number of strips from the pouch and fit them to the frame provided. Unused strips should be returned to the pouch and stored at 2-8°C.

7.2. Prepare Washing Buffer as detailed in section 5.1.

7.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.

7.4. Pipette 0.1ml of Negative Control (Green label) into the second well.

7.5. Pipette 0.1ml of Positive Control (Red label) into the third well.

7.6. Pipette 0.1ml of each boiled/cooled sample separately into consecutive wells in the strip. If there are wells left over at the end of a test strip the Positive or Negative Controls may be repeated.

7.7. Incubate the plate (containing the strips) at 37±1°C for 30 mins (±5 mins).

7.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5-7 times with Washing buffer ensuring complete filling and emptying of the wells through each wash cycle. The washing technique is critical to assay performance, hence it is recommended to use a microplate washer.

7.9. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.

7.10. Incubate the plate at 37±1°C for 30 mins (±5 mins).

7.11. Repeat the wash cycles as detailed in section 7.9.

7.12. Pipette 0.1ml of TMB Substrate (Blue label) into all wells, including the 'blank' well.

7.13. Incubate the plate at room temperature for 30 mins. (±5 mins).

7.14. After incubation stop the reaction by adding 0.1ml of Stop Solution (Silver label) to all wells including the 'blank' well. The Stop Solution will cause any blue colour in the wells to change to yellow.

7.15. Read the optical densities within 10 minutes in a plate reader using a 450nm filter (do not use reference filter). Inspect the wells before reading for air bubbles and if present burst with a needle. Zero the reader against the 'blank' well before the other wells are read.

8. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD₄₅₀) measurements using microplate reader. Subtract the OD value of the blank well (usually A1) from all of the other results. Assay acceptance criteria:

Assay acceptance criteria:

Negative Control OD ₄₅₀	< 0.100
Positive Control OD ₄₅₀	> 0.500

Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be repeated. Samples with OD₄₅₀ readings of less than 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-heat treated samples may be discarded following local regulations/guidelines.

Sample wells with OD₄₅₀ \geq 0.200 are considered presumptive positive for *Salmonella*. Presumptive positive results must be verified using a recognised culture method.

9. CONFIRMATION OF POSITIVE RESULTS FROM SALMONELLA IMMUNOASSAY

All samples identified as positive by the alternative method must be confirmed in one of the following ways.

The confirmation step must start from the non-heat treated samples stored at 41.5°C or 2-8°C:

Streak the supplemented BPW sample onto 1 agar plate (XLD or a chromogenic agar for *Salmonella* such as Colorex *Salmonella* from Chromagar). Incubate agar as specified by standard *Salmonella* cultural protocols and then perform confirmation tests e.g. Microgen latex test F42 or biochemical identification gallery directly on isolated colonies without purification step or by performing the tests described in the standardised methods (CEN or ISO). The F42 latex test uses polyclonal antibody to detect flagellar antigens. It is not adapted for the detection of non-motile *Salmonella*.

Or additionally subculture the supplemented BPW sample (0.1ml +10ml) in RVS broth incubate for 24h +/-3h at 41.5°C +/-1°C. Streak onto XLD or a chromogenic agar for *Salmonella* such as Colorex *Salmonella* from Chromagar). Incubate agar as specified by standard *Salmonella* cultural protocols and then perform confirmation tests e.g. Microgen latex test F42 or biochemical identification gallery directly on isolated colonies without purification step or by performing the tests described in the standardised methods (CEN or ISO).

In the event of discordant results (presumptive positive ELISA results, not confirmed by one of the means described above and in particular the latex test) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

10. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. DO NOT FREEZE. The kit expiry date is displayed on the kit box plus all of the kit components.

11. SAFETY

The Stop Solution contains sulphuric acid which is corrosive. Wash immediately with large quantities of water if the solution comes into contact with skin or mucous membranes.

For use in laboratory facilities with trained personnel for the handling of potentially pathogenic organisms. Training is recommended to first time users and can be provided by Solus Scientific.

As a guide, the following precautions should be taken as a minimum:

- Protective clothing should be worn including safety glasses, laboratory coat and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with skin.

12. PRECAUTIONS

- Reagents are provided at a matched working concentration. Optimum sensitivity and specificity will be reduced if reagents are modified or not stored under the recommended conditions.
- Do not mix different lots of reagents.
- Avoid microbial contamination of opened reagent bottles.
- Ensure that no cross contamination occurs between any of the reagents or wells.
- Ensure that the Conjugate does not contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- Avoid using solutions containing sodium azide for cleaning of equipment especially washing devices (the peroxidase enzyme used in the kit is inactivated by sodium azide).
- Do not use for diagnostic purposes with medical specimens.

13. SDS INFORMATION

Safety data sheets (SDS) are available for this test on request.

14. WARRANTY

Accurate results depend on the proper use of the kit by following the instructions for use carefully. If the kit fails to perform according to specification please contact Solus Scientific.



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