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Food safety excellence

Solus One *Salmonella*

Immunoassay
Based Test System
For the Detection
of *Salmonella*
in Foods and
Environmental
Samples

www.solusscientific.com



Solus Scientific Solutions Ltd.

9 Mansfield Networkcentre,
Millennium Business Park, Concorde Way,
Mansfield, Nottinghamshire NG19 7JZ
UK

Tel - +44 (0)1623 429701

Fax - +44 (0)1623 620977

Email: info@solusscientific.com



T: 02 8212 4074 F: 02 9423 6992

info@keydiagnostics.com.au

www.keydiagnostics.com.au

PO Box 1038, Gymea, NSW, 2227

Product Number: SAL1-0480

Solus One *Salmonella* is for the next day detection of *Salmonella* in foods and production environment samples.

**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 qualified and trained personnel. Basic training is recommended to first time users and can be provided by Solus Scientific. Using the method requires 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 with only the Washing Buffer Activator and Washing Buffer reagent requiring dilution.

The contents of an activator sachet must be first dissolved in 1440ml of deionised (DI) water followed by the addition of the 60ml concentrated Washing Buffer reagent to this solution.

Each kit contains sufficient material for 5 x 93 determinations, plus controls.

The kit expiry date is displayed on each product label.

- 5 x 96 well microplates (in breakable strip format). Wells are coated with antibodies against *Salmonella* spp.
- Negative Control (Green label). 10ml in working dilution. Contains diluent with preservative.
- Positive Control (Red label). 10ml in working dilution. Contains heat-killed *Salmonella* in diluent with preservative. The positive control is black in colour.
- Conjugate (Orange label). 60ml in working dilution. Contains horseradish peroxidase-antibody conjugate in diluent with preservative.
- Substrate (Blue label). 60ml in working dilution. Contains 3,3',5,5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers. Solution should be colourless.
- Stop Solution (Silver label). 60ml in working dilution. Contains 0.2M sulphuric acid.
- Washing Buffer Concentrate (25x). 5 x 60ml.
- Washing Buffer Activator. 5 x 1 Sachet.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C
- Deionised or distilled water
- MED017 - Buffered Peptone Water (BPW) according to ISO 6579
- SALSUPP-112 – Solus One *Salmonella* Supplement
- 70% v:v Ethanol

- Measuring cylinder for 250ml or 1L
- Filter bags (for food samples)
- Sterile 10ml tubes suitable for selective enrichment culture (for swabs)
- Sponge swabs soaked in suitable neutralising buffer (e.g. Lethen broth or HiCap buffer - World Bioproducts)
- FRI001 - frit filters
- Homogeniser
- 3ml transfer pipettes (sterile)
- Tube for sample boiling (e.g. 5ml Polypropylene rimless tubes 12x75mm)
- Vortex mixer
- Timer
- Incubator or water bath at 41.5±1°C
- Heating block or water bath (capable of heating to 85-100°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 2 litre vessel –

5.1.1 Add the contents of a Washing Buffer Activator Sachet to 1440ml DI water and mix until the activator has fully dissolved.

5.1.2 Add 60ml of the concentrated washing buffer reagent to the vessel containing 1440ml of the dissolved activator solution.

5.2. Culture Broth (growth medium): Prepare Buffered Peptone Water (BPW) ISO 6579 following manufacturer's instructions.

5.3. Prepare the Solus One *Salmonella* Supplement according to the manufacturer's instructions. Add 4.44ml of supplement per 1L (1ml per 225ml) of BPW

6. SAMPLE PREPARATION AND ENRICHMENT- standard method**Food samples****1 in 10 dilution:**

25g sample - Homogenise 25g of the sample in 225ml of supplemented BPW, and incubate for 20 to 22 hours at 41.5±1°C.

100g sample - Warm supplemented BPW to 37°C prior to use. Homogenise 100g of the sample by hand massaging in 900ml of pre-warmed supplemented BPW, and incubate for 20 to 22 hours at 41.5±1°C.

1 in 4 dilution:

375g sample - Warm supplemented BPW to 37°C prior to use. Homogenise 375g of the sample by hand massaging in 1125ml of pre-warmed supplemented BPW, and incubate for 20 to 22 hours at 41.5±1°C.

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

Environmental samples

Sample surface with sampling device. Follow sampling device manufacturers' instructions for correct use, storage and transport.

Warm supplemented BPW to 37°C prior to use. Add 100ml of pre-warmed, supplemented BPW to sponge sampler (e.g. World bioproducts or Hygiena) in a suitable bag. Add 10ml of pre-warmed, supplemented BPW to a swab (e.g. sponge or foam). Hand massage or mix by vortexing and incubate for 16 to 20 hours at 41.5±1°C.

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 with dessicant 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 eat treated 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 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 aspiration and wash cycles as detailed in section 7.8.

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 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 a 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 a 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 sample may be discarded following local regulations/guidelines.

Sample wells with OD₄₅₀ ≥ 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

Assay positive samples may be confirmed by cultural methodology described in standardised methods such as FDA (BAM), USDA MLG or ISO.

Sub-culture the non-heat treated sample onto two selective agars and a selective enrichment broth. Incubate as appropriate. Following incubation examine the plates for the presence of typical colonies. If typical colonies are observed confirm by use of appropriate techniques.

If no typical colonies are observed sub culture the selective enrichment onto two selective agars and incubate as appropriate. Following incubation examine the plates for the presence of typical colonies. If typical colonies are observed confirm by use of appropriate techniques. If no typical colonies are observed the sample is regarded as negative.

Only trained microbiologists should attempt confirmation.

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.
- 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 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.