

Issue number: **4.0**

Date: **12/19**

Product code: **SAL1-0480**



Food safety excellence

Solus **One** *Salmonella*

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

www.solusscientific.com

1. INTRODUCTION

Solus One *Salmonella* is for the next day detection of *Salmonella* in selected foods and production environment samples. This assay provides a negative or a presumptive positive result from a single enrichment step within 24 hours, including the assay time.

2. INTENDED USE

AOAC applicability is based on a validation study for selected foods (raw beef trim, pasteurized liquid egg, raw salmon, cheddar cheese, romaine lettuce, instant non-fat dairy milk powder, whole black peppercorns, paprika powder, cinnamon powder, honey mustard onion seasoning, flavored ranch seasoning, cocoa powder, cocoa liquor, milk chocolate bar), sponge samplers with stainless steel environmental surfaces and swabs with plastic surfaces.

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

3. REAGENTS PROVIDED

Most kit components are supplied stabilized and ready to use at working concentration with only the Washing Buffer Activator and Washing Buffer reagent requiring dilution. As part of real-time stability trials, a 12 month shelf-life has been assigned to the Solus One *Salmonella* kit components.

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 color.
- 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 stabilizers. Solution should be colorless.
- Stop Solution (Silver label). 60ml in working dilution. Contains 0.2M sulfuric acid.

- Washing Buffer Concentrate (25x). 5 x 60ml.
- Washing Buffer Activator. 5 x 1 sachet. The contents of an activator sachet must be first dissolved in 1440ml of deionized (DI) water followed by the addition of the 60ml concentrated Washing Buffer reagent to this solution.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Refrigerator at 2-8°C
- Deionized or distilled (DI) water
- Buffered Peptone Water (BPW)
- Solus Modified Buffered Peptone Water (Solus mBPW)
- Solus One *Salmonella* Supplement
- 70% v:v Ethanol
- Measuring cylinder for 2L
- Filter bags for food samples
- Sterile 10ml tubes suitable for selective enrichment culture (for swabs)
- Sponge swabs soaked in suitable neutralizing buffer (e.g. Lethen broth or HiCap buffer - World Bioproducts)
- Frit filters
- Homogenizer
- 3ml transfer pipettes (sterile)
- Precision pipettes – For sampling and delivering of 100–300µl, and 200–1,000µl
- Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)
- Vortex mixer
- Timer
- Incubator at 41.5±1°C
- Heating apparatus (e.g. heat block) 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

Washing Buffer

Prepare the following in a clean vessel.

- 5.1. Add the contents of a Washing Buffer Activator Sachet to 1440ml DI water and mix until the activator has fully dissolved.
- 5.2. Add 60ml (1 bottle) of the concentrated Washing Buffer reagent to the vessel containing 1440ml of the dissolved activator solution.

Culture Broth (growth medium)

- 5.3. Prepare Buffered Peptone Water (BPW) and/or Solus Modified Buffered Peptone Water (Solus mBPW) as required following manufacturer's instructions. Allow to cool to ambient temperature (e.g. 18 to 25°C) before use.
- 5.4. Reconstitute the Solus One *Salmonella* Supplement according to the manufacturer's instructions.
- 5.5. Supplement culture broth (BPW or Solus mBPW) to the level appropriate for the matrix to be tested. See section 6 for details. Ensure BPW/Solus mBPW is pre-warmed prior to addition of Solus One *Salmonella* Supplement where required.

6. SAMPLE PREPARATION AND ENRICHMENT- STANDARD METHOD

Food samples

1 in 10 dilution:

25g sample (Raw salmon (fillet), cheddar cheese (shredded), romaine lettuce (bagged)) - Homogenize 25g of the sample in 225ml of BPW supplemented at 4.44ml/L, and incubate for 20 to 22 hours at 41.5±1°C.

100g sample (Pasteurized liquid egg) - Warm BPW to 37±2°C prior to supplementing at 4.44ml/L for use. Homogenize 100g of the sample by automation or hand massaging for 2 minutes 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 (Raw beef trim) - Warm BPW to 37±2°C prior to supplementing at 4.44ml/L for use. Homogenize 375g of the sample by automation or hand massaging for 2 minutes in 1125ml of pre-warmed & supplemented BPW, and incubate for 20 to 22 hours at 41.5±1°C.

375g sample (Instant non-fat dairy milk powder) - Warm BPW to 37±2°C prior to supplementing at 4.44ml/L for use. Homogenize 375g of the sample by

automation or hand massaging for 2 minutes in 1125ml of pre-warmed & supplemented BPW, and incubate for 21 to 22 hours at $41.5\pm 1^{\circ}\text{C}$.

Herbs and Spices

1 in 10 dilution:

25g sample (Paprika powder, whole black peppercorns). Homogenize 25g of the sample in 225ml of Solus mBPW supplemented at 2.22ml/L and incubate for 20 to 24 hours at $41.5\pm 1^{\circ}\text{C}$.

375g sample (Honey mustard onion seasoning, flavored ranch seasoning). Homogenize 375g of the sample in 3375ml of Solus mBPW supplemented at 2.22ml/L and incubate for 20 to 24 hours at $41.5\pm 1^{\circ}\text{C}$.

1 in 50 dilution:

25g sample (Cinnamon powder) Homogenize 25g of the sample in 1225ml of Solus mBPW supplemented at 2.22ml/L and incubate for 20 to 24 hours at $41.5\pm 1^{\circ}\text{C}$.

Confectionery

1 in 10 dilution:

25g sample (Cocoa powder, cocoa liquor, milk chocolate bar). Homogenize 25g of the sample in 225ml of Solus mBPW supplemented at 2.22ml/L and incubate for 20 to 24 hours at $41.5\pm 1^{\circ}\text{C}$.

375g sample (Cocoa powder, cocoa liquor, milk chocolate bar). Homogenize 375g of the sample in 3375ml of Solus mBPW supplemented at 2.22ml/L and incubate for 20 to 24 hours at $41.5\pm 1^{\circ}\text{C}$.

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 BPW to $37\pm 2^{\circ}\text{C}$ prior to supplementing at 4.44ml/L for 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 for 2 minutes or mix by vortexing for 2 minutes and incubate for 16 to 20 hours at $41.5\pm 1^{\circ}\text{C}$.

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

7. POST ENRICHMENT HEAT INACTIVATION

- 7.1. Turn on heating apparatus to bring up to working temperature (85-100°C) prior to initiating testing.
- 7.2. When the sample incubation period is completed, carefully remove a 1-2ml aliquot, avoiding particulate matter, to a sample boiling tube (e.g. polypropylene tube).
- 7.3. Heat the aliquot at 85-100°C for 15-20 minutes in the tube. After heating allow the sample to cool to room temperature (18-25°C). This may be accelerated by placing the tubes in cold water for 5 minutes.
- 7.4. Keep the non-heat treated samples for verification until immunoassay results are obtained. These samples can be kept at $41.5\pm 1^{\circ}\text{C}$ 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.

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

NOTE - The frit should only be inserted after the heating stage, and once the tube has cooled back to room temperature (18-25°C).

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

8. IMMUNOASSAY PROCEDURE

- 8.1. Take the test kit from storage at 2-8°C one hour before use to allow the components to reach room temperature (18-25°C). 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 desiccant and stored at 2-8°C.
- 8.2. Prepare Washing Buffer as detailed in section 5.
- 8.3. Leave the first well in the strip empty to serve as a 'blank' for measuring the absorbance of the substrate.

- 8.4. Pipette 0.1ml of Negative Control (Green label) into the second well.
- 8.5. Pipette 0.1ml of Positive Control (Red label) into the third well.
- 8.6. Pipette 0.1ml of each heat 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.

If using the Dynex instrumentation, care must be taken to avoid bubbles in the sample and reagent tubes, or "films" forming across the tube above the level of the liquid. It is essential to check that the system has successfully pipetted samples into the assay plate before proceeding.

- 8.7. Incubate the plate at $37\pm 1^{\circ}\text{C}$ for 30 minutes (± 5 mins).
- 8.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible.
- 8.9. 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.
- 8.10. Pipette 0.1ml of Conjugate (Orange label) into all wells except the 'blank'.
- 8.11. Incubate the plate at $37\pm 1^{\circ}\text{C}$ for 30 minutes (± 5 mins).
- 8.12. Repeat the aspiration and wash cycles as detailed above.
- 8.13. Pipette 0.1ml of Substrate (Blue label) into all wells, including the 'blank' well.
- 8.14. Incubate the plate at room temperature ($18\text{-}25^{\circ}\text{C}$) for 30 minutes (± 5 mins).
- 8.15. 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 color in the wells to change to yellow.
- 8.16. Read the optical densities of the wells within 10 minutes in a plate reader using a 450nm filter (do not use a reference filter). Inspect the wells for air bubbles before reading and if present burst with a needle. Zero the reader against the 'blank' well before the other wells are read.

Assay acceptance criteria:

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

9. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD₄₅₀) measurements using a microplate reader.

Subtract the OD₄₅₀ value of the blank well from all of the other results.

Should the value of Negative or Positive controls not meet these criteria, the test is not considered valid and must be performed again.

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.

Samples with OD₄₅₀ ≥ 0.200 are considered presumptive positive for *Salmonella*. Presumptive positive results must be verified using a recognized culture method.

10. CONFIRMATION OF POSITIVE RESULTS FROM SALMONELLA IMMUNOASSAY

Assay positive samples may be confirmed by cultural methodology described in standardized methods such as the U.S. Food and Drug Administration Bacteriological Analytical Manual (BAM) Chapter 5: *Salmonella*, U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Manual (MLG) 4.09 or International Standards Organization (ISO) 6578-1.

Subculture the non heat-treated sample onto two selective agars and into 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, subculture 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.

11. KIT STORAGE AND EXPIRY

The kit and any unused kit components should be stored at 2-8°C. The kit expiry date is displayed on the kit box plus all of the kit components.

DO NOT FREEZE.

12. SAFETY

Solus One *Salmonella* method

- Ensure that the heating apparatus reaches a temperature of 85–100°C and the sample is heated for 15–20 minutes thus ensuring the organisms are killed and the sample is safe to handle.
- The Stop Solution contains sulfuric 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 qualified and trained personnel for the handling of potentially pathogenic organisms.
- Training is recommended to first time users and can be provided by Solus Scientific Solutions Ltd.

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.

Dynex DS2 - Improper use of the Dynex DS2 may cause personal injury or damage to the instrument. For safe use, the Dynex DS2 must be operated only by qualified laboratory personnel who have been appropriately trained.

Enrichment –*Salmonella* is a Biosafety Level 2 organism. Biological samples such as enrichments have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes but is not limited to: protective eyewear, face shield, clothing/laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (e.g. physical contaminant devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture based confirmatory steps through heat denaturation by autoclaving at 121°C for 15 minutes.

13. PRECAUTIONS FOR OPTIMAL PERFORMANCE

- Most kit components are supplied stabilized and ready to use at working concentration with only the Washing Buffer Activator and Washing Buffer reagent requiring dilution. 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 wells.
- Ensure that the Conjugate does not contaminate other reagents or equipment.
- Ensure the Substrate is not exposed to bright light for extended periods of time.
- 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.

14. SAFETY DATA SHEETS

Safety data sheets are available from our website at www.solusscientific.com

15. 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:

Tel: +44 (0)1623 429701
Fax: +44 (0)1623 620977
Email: info@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



PERFORMANCE TESTED

AOAC
RESEARCH INSTITUTE

LICENSE NUMBER 101801