SOLUS ONE *SALMONELLA*

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

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Certifying Body: AOAC

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This method's performance was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

This method has been evaluated in the AOAC® *Performance Tested Methods*℠ Program for the detection of *Salmonella* spp. in raw salmon fillet, bagged romaine lettuce, shredded cheddar cheese, instant non-fat dry milk, pasteurized liquid eggs (100g), raw beef trim (375g), honey mustard seasoning, flavored ranch seasoning, cinnamon powder, paprika powder, whole black peppercorns, cocoa powder, cocoa liquor, milk chocolate bar, stainless steel and polystyrene environmental surfaces. Solus One *Salmonella* method has been compared to the USDA-FSIS MLG 4.09 and FDA/BAM Chapter 5 reference methods, as applicable to matrices. (AOAC® *Performance Tested*℠ License no. **101801**).

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1. INTRODUCTION

Solus One *Salmonella* provides a negative or a presumptive positive result from a single enrichment step within 24 hours, including the assay time.

2. INTENDED USE

Solus One *Salmonella* is for the next day detection of *Salmonella* spp. in selected foods and production environmental samples. The test method is easy to perform, however it requires laboratory facilities plus qualified and trained personnel. Basic training is recommended to first time users and is given by Solus Scientific Solutions Ltd.

Using the method includes 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 concentrate requiring dilution. Each kit contains sufficient material for 1 (SAL1-0096) or 5 (SAL1-0480) x 93 determinations, plus controls. The kit expiry date is displayed on each product label.

Component	Appearance	Volume		Comments
		SAL1-0096	SAL1-0480	
Assay plate	96-well microplate with removable/ breakable strip format	1	5	Wells coated with antibodies against *Salmonella* spp.
Negative control	Pale orange liquid. Green label.	3ml	10ml	Working concentration. Contains diluent with preservative.
Positive control	Black liquid. Red label.	3ml	10ml	Working concentration. Contains heat-killed *Salmonella* in diluent with preservative.
Conjugate	Colorless/very pale straw-colored liquid. Orange label.	11ml	60ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colorless/very pale blue liquid. Blue label.	11ml	60ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilizers.
Stop solution	Colorless liquid. Silver label.	11ml	60ml	Working concentration. Contains 0.2M sulfuric acid.
Washing buffer concentrate	Colorless/yellow/orange liquid. White label.	10ml x 6	60ml x 5	Concentrated. Dilute before use.
Washing buffer activator	Foil sachet of white granular powder.	6 (small)	5 (large)	Washing buffer activator must be dissolved in required volume of deionized (DI) water prior to addition of the washing buffer concentrate to the solution. See section 5 for details.
Frit filters	Flat, white plastic discs ~12mm diameter	100	500	Frit filters should be applied to each sample tube after heat-treatment once the sample has cooled back to ambient temperature (18-25°C). See section 7 for details.

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4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Refrigerator at 2-8°C	3ml transfer pipettes (sterile)
Deionized or distilled (DI) water	Vortex mixer
Buffered Peptone Water (BPW)	Timer
Solus Modified Buffered Peptone Water (Solus mBPW)	Incubator at 37±1°C
Solus One Supplement	Incubator at 41.5±1°C
70% v:v Ethanol	Tubes for sample boiling (e.g. 5ml polypropylene rimless tubes 12x75mm)
Filter bags	
Sponge samplers or swabs soaked in suitable neutralizing buffer (e.g. Letheen broth or HiCap buffer)	Heating apparatus (e.g. heat block) capable of heating to 85-100°C
Homogenizer (or similar apparatus)	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
Pipettes and tips (1ml; 0.1ml)	
Measuring cylinders for various volumes (e.g. 250 ml, 1L)	Autoclave for decontamination of samples

5. REAGENT PREPARATION

5.1 Wash Buffer:

Prepare the following in a clean vessel.

SAL1-0096	SAL1-0480
Add the contents of 1 x washing buffer activator sachet to 240ml DI water. Mix until the activator has fully dissolved.	Add the contents of 1 x washing buffer activator sachet to 1440ml DI water. Mix until the activator has fully dissolved.
Add 10ml (1 bottle) of the washing buffer concentrate to the vessel containing the activator solution and swirl to mix.	Add 60ml (1 bottle) of the washing buffer concentrate to the vessel containing the activator solution and swirl to mix.

5.2 Culture Broth (growth medium):

- Prepare Buffered Peptone Water (BPW) and/or Solus Modified Buffered Peptone Water (Solus mBPW) following manufacturer's instructions. Allow to cool to ambient temperature (e.g. 18-25°C) before use.
- Reconstitute the Solus One Supplement according to the manufacturer's instructions. Prepare supplemented culture broth (BPW or Solus mBPW) at the level appropriate to the supplement pack size being used and the strength required for the matrix to be tested. See section 6 for details.

	SALSUPP22.5 or SALSUPP112.5	SALSUPP200
"Full strength"	Add 4.44ml of supplement per 1L of culture broth	Add 2.5ml of supplement per 1L of culture broth
"Half strength"	Add 2.22ml of supplement per 1L of culture broth	Add 1.25ml of supplement per 1L of culture broth

NOTE: Ensure BPW/Solus mBPW is pre-warmed prior to addition of Solus One Supplement where required as per section 6.

6. SAMPLE PREPARATION AND ENRICHMENT

6.1 Food Samples

1 in 10 dilution in full strength supplemented BPW:

- 25g sample (Raw salmon, cheddar cheese, romaine lettuce) - Homogenize 25g of the sample in 225ml of BPW supplemented at full strength and incubate for 20-22 hours at 41.5±1°C.
- 100g sample (Pasteurized liquid egg) - Warm BPW to 37±2°C prior to supplementing at full strength for use. Homogenize 100g of the sample in 900ml of pre-warmed & supplemented BPW and incubate for 20-22 hours at 41.5±1°C.

1 in 4 dilution in full strength supplemented BPW:

- 375g sample (Raw beef trim) - Warm BPW to 37±2°C prior to supplementing at full strength for use. Homogenize 375g of the sample in 1125ml of pre-warmed & supplemented BPW and incubate for 20-22 hours at 41.5±1°C.
- 375g sample (Instant non-fat dry milk powder) - Warm BPW to 37±2°C prior to supplementing at full strength for use. Homogenize 375g of the sample in 1125ml of pre-warmed & supplemented BPW and incubate for 21-22 hours at 41.5±1°C.

6.2 Herbs and Spices

1 in 10 dilution in half strength supplemented Solus mBPW:

- 25g sample (Paprika powder, whole black peppercorns). Homogenize 25g of the sample in 225ml of Solus mBPW supplemented at half strength and incubate for 20-24 hours at 41.5±1°C.
- 375g sample (Honey mustard onion seasoning, flavored ranch seasoning). Homogenize 375g of the sample in 3375ml of Solus mBPW supplemented at half strength and incubate for 20-24 hours at 41.5±1°C.

1 in 50 dilution in half strength supplemented Solus mBPW:

- 25g sample (Cinnamon powder) Homogenize 25g of the sample in 1225ml of Solus mBPW supplemented at half strength and incubate for 20-24 hours at 41.5±1°C.

6.3 Confectionery

1 in 10 dilution in half strength supplemented Solus mBPW:

- 25g sample. Homogenize 25g of the sample in 225ml of Solus mBPW supplemented at half strength and incubate for 20-24 hours at 41.5±1°C.
- 375g sample (Cocoa powder, cocoa liquor, milk chocolate bar). Homogenize 375g of the sample in 3375ml of Solus mBPW supplemented at half strength and incubate for 20-24 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.

6.4 Environmental samples

Full strength supplemented BPW:

- Sample the production environment surface with sampling device. Follow sampling device manufacturer's instructions for correct use, storage and transport.
- Warm BPW to 37±2°C prior to supplementing at full strength for use. Add 100ml of pre-warmed & supplemented BPW to sponge sampler in a suitable sample bag. Add 10ml of pre-warmed & supplemented BPW to swabs. Hand massage for 2 minutes (sponge samplers) or mix by vortexing for 2 minutes (swabs) and incubate for 16-20 hours at 41.5±1°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. When the sample incubation period is completed, transfer 1-2ml aliquot (avoiding particulates) to a sample boiling tube (e.g. 5ml polypropylene tube).

7.2. Heat the aliquot to 85-100°C for 15-20 minutes in the tube. After heating, allow the sample to cool to ambient temperature (18-25°C). This may be accelerated by placing the tubes in cold tap water for ~5 minutes.

7.3. To prevent pipetting issues, especially on the Dynex DS2 instrument, add a frit filter directly into each sample tube and gently push down to allow 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 ambient 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 egg-based products and caseinates. These samples may require manual addition to the immunoassay.

The non-heat-inactivated samples should be kept for verification until immunoassay results are obtained. These samples should be kept at 41.5±1°C if the test is to be carried out within 2 hours. If this is not possible, keep the broths for up to 72 hours at 2-8°C prior to the test.

8. IMMUNOASSAY PROCEDURE

8.1. Take test kit from storage at least one hour before use to allow the components to reach ambient temperature (18-25°C). Determine the number of wells required for the test. Take 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.

8.2. Prepare Wash Buffer as detailed in section 5.1 for the kit size being used.

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-inactivated 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. †

8.7. Incubate the plate (containing the strips) at 37±1°C for 30-35 minutes.

8.8. After incubation, aspirate the contents of the wells, removing as much of the liquid as possible. Wash the wells 5 times with wash 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 instrument.

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

8.10. Incubate the plate at 37±1°C for 30-35 minutes.

8.11. Repeat the wash cycles as detailed in section 8.8

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

8.13. Incubate the plate at ambient temperature (18-25°C) for 30-35 minutes in the dark.

8.14. 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 color in wells to change to yellow.

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8.15. Read the optical densities of wells within 10 minutes in a plate reader using a 450nm filter. Before reading, inspect the wells for air bubbles and, if present, burst with a needle. The reader should be zeroed against the 'blank' well before the other wells are read. Do not use reference filter. The use of automatic ELISA equipment is preferred and should be set up and validated to this protocol.

† 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.

9. INTERPRETATION OF RESULTS

Results are expressed as optical density (OD_{450}) measurements using a microplate reader.

Acceptance criteria:

Negative Control OD_{450}	< 0.100
Positive Control OD_{450}	> 0.500

The value of the blanking well (usually A1 when processing manually) should always be subtracted from all 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 OD_{450} readings of < 0.200 are considered negative in which case the analysis is complete, the results may be reported and the corresponding non-heat-inactivated samples may be discarded following local regulations/ guidelines.

Sample with OD_{450} ≥ 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 ONE *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-inactivated 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.

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11. 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 within the box. Any unused diluted wash buffer can be stored for up to 10 days if kept at 2-8°C. Any unused microplate strips should be returned to the foil pouch with the desiccant sachet and the seal closed completely, then stored at 2-8°C.

12. SAFETY

While the procedures detailed are simple and easy to perform, they require laboratory facilities with qualified personnel trained for the handling of potentially pathogenic organisms. Training is recommended to first time users and is provided by Solus Scientific Solutions Ltd.

- 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.

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

- Protective clothing should be worn including lab coat, safety glasses, mask and gloves where appropriate.
- Do not pipette by mouth.
- Avoid contact with the skin.
- Do not eat, drink or apply cosmetics in the laboratory.
- Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes.

13. PRECAUTIONS FOR OPTIMAL PERFORMANCE

- Reagents are provided at fixed 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 wells.
- It is essential for proper performance of the test that the enzyme-conjugated antibody is not allowed to contaminate other reagents and equipment.
- Ensure that kit components are not exposed to temperatures greater than 40°C.
- Solutions containing sodium azide should not be used 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 of medical specimens.

14. MSDS INFORMATION

Material safety data sheets (MSDS) are available for this test on request.

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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:

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Summary of changes

Change date	Issue Number	Change Summary
Jan2020	1	Rebrand and combining of Solus One Salmonella – AOAC – Insert 30 – Issue 4 – 12/19 and Solus One Salmonella – AOAC – Insert 31 – Issue 4 – 12/19

NOTE: Minor changes (e.g. formatting, grammar, correcting typographical errors) are not included in the summary of changes.

For more information visit www.solusscientific.com

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