

# SOLUS ONE *SALMONELLA*

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

**Insert Number:** 39  
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**Certifying Body:** AFNOR





SOL 37/04-12/18  
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS  
<http://nf-validation.afnor.org/en>

This method is certified by AFNOR Certification for the detection of *Salmonella* in the following categories: ready-to-eat, ready-to-reheat (excluding smoked products), heat processed milk and dairy products, egg products meat products (raw meat products, raw poultry and cured products (validation ref. no. **SOL 37/04-12/18**).

## 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. 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

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 <i>Salmonella</i> 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 <i>Salmonella</i> in diluent with preservative.
Conjugate	Colourless/very pale straw-coloured liquid. Orange label.	11ml	60ml	Working concentration. Contains horseradish peroxidase antibody conjugate in diluent with preservative.
Substrate	Colourless/very pale blue liquid. Blue label.	11ml	60ml	Working concentration. Contains 3, 3', 5, 5'-Tetramethylbenzidine (TMB), hydrogen peroxide and stabilisers.
Stop solution	Colourless liquid. Silver label.	11ml	60ml	Working concentration. Contains 0.2M sulphuric acid.
Washing buffer concentrate	Colourless/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 deionised (DI) water prior to addition of the washing buffer concentrate to the solution. See section 5.1 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.

Most kit components are supplied stabilised 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.



## 4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Refrigerator at 2-8°C	Vortex mixer
Deionised or distilled (DI) water	Timer
Buffered Peptone Water (BPW)	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)
Measuring cylinders for various volumes (e.g. 250 ml, 1L)	Heating apparatus (e.g. heat block) capable of heating to 85-100°C
Filter bags	Pipettes and tips (1ml; 0.1ml)
Homogeniser (or similar apparatus)	Dynex DS2 or Microplate washer and microplate reader with 450nm filter
3ml transfer pipettes (sterile)	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) following manufacturer's instructions. Allow to cool to ambient temperature (18-25°C) before use in testing.
- Reconstitute the Solus One Supplement according to the manufacturer's instructions. Prepare supplemented BPW appropriate to the supplement pack size being used.

SUPPLEMENT LEVEL	SALSUPP22.5; SALSUPP112.5	SALSUPP200
Full-strength	Add 4.44ml of supplement per 1L of BPW Add 1ml of supplement per 225ml of BPW	Add 2.5ml of supplement per 1L of BPW Add 0.56ml of supplement per 225ml of BPW
Half-Strength	Add 2.22ml of supplement per 1L of BPW Add 0.5ml of supplement per 225ml of BPW	Add 1.25ml of supplement per 1L of BPW Add 0.28ml of supplement per 225ml of BPW

## 6. SAMPLE PREPARATION AND ENRICHMENT

### 6.1 Standard Method

- Homogenise 25g of the test sample (ready-to-eat, ready-to-reheat, heat processed milk and dairy products, and egg products) in 225ml of full-strength supplemented BPW and incubate for 20-22 hours at 41.5±1°C. Some enriched sample types can contain particulates that make pipetting difficult. We recommend use of filter bags for enrichment to contain the particulates and reduce pipetting issues



## 6.2 Specific Method

- Homogenise 25g of the test sample (meat products) in 225ml of half-strength supplemented BPW and incubate for 22-24 hours at  $41.5 \pm 1^\circ\text{C}$ . Some enriched sample types can contain particulates that make pipetting difficult. We recommend use of filter bags for enrichment to contain the particulates and reduce pipetting issues.

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. Ensure that the bench processing time of samples is kept to a minimum and that transfer to  $41.5^\circ\text{C}$  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  $85-100^\circ\text{C}$  for 15-20 minutes in the tube. After heating, allow the sample to cool to ambient temperature ( $18-25^\circ\text{C}$ ). 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 ( $18-25^\circ\text{C}$ ).

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  $41.5 \pm 1^\circ\text{C}$  if the immunoassay test is to be carried out within 2 hours. If this is not possible, keep the broths for up to 72 hours at  $2-8^\circ\text{C}$  prior to the immunoassay 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 ( $18-25^\circ\text{C}$ ). 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  $2-8^\circ\text{C}$ .
- 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  $37 \pm 1^\circ\text{C}$  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  $37\pm 1^{\circ}\text{C}$  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 ( $18-25^{\circ}\text{C}$ ) for 30 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 colour in wells to change to yellow.
- 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 ( $\text{OD}_{450}$ ) measurements using a microplate reader.

Acceptance criteria:

Negative Control $\text{OD}_{450}$	< 0.100
Positive Control $\text{OD}_{450}$	> 0.500

The value of the blanking well (usually A1 when processing manually) should always be subtracted. 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  $\text{OD}_{450}$  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 wells with  $\text{OD}_{450} \geq 0.200$  are considered presumptive positive for *Salmonella*. Presumptive positive results must be verified using a recognised culture method.

## 10. CONFIRMATION OF POSITIVE RESULTS FROM ONE SALMONELLA IMMUNOASSAY

In the context of NF VALIDATION, all samples identified as positive by the alternative method must be confirmed by one of the following tests:

- Using the conventional tests described in the standardised methods by CEN or ISO. The confirmation step must start from the non-heat-inactivated supplemented BPW sample stored at  $41.5^{\circ}\text{C}$  or  $2-8^{\circ}\text{C}$ . Streak supplemented BPW sample (10 $\mu\text{L}$ ) onto 1 agar plate (XLD or a chromogenic agar for



*Salmonella* such as Colorex *Salmonella* from Chromagar). Incubate agars as specified by standard *Salmonella* cultural protocols then perform confirmation tests: latex test F42 from Microgen or biochemical identification gallery.

- Or additionally subculture the non-heat-inactivated sample (0.1ml+10ml) in RVS broth and incubate for 21-27h 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).

NOTE: The F42 latex test uses polyclonal antibodies to detect flagella antigens, it is not adapted for the detection of non-motile *Salmonella*. It is possible to perform confirmation tests directly if the colonies are well isolated.

In the event of discordant results (presumptive positive immunoassay result non-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.

## 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.

- 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.

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

- 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.

### 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	Rebranding and combining of Solus One Salmonella – insert 28 – Issue 1.1 – 09/19 and Solus One Salmonella – insert 21 – Issue 1.2 – 12/18 into a single document.
March 2021	2	Addition of meat products to categories on AFNOR certification page, addition of supplement table at both half-strength and full-strength supplement levels and addition of specific method for meat products.

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](http://www.solusscientific.com)

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