BAC*Spec Salmonella* 2

ELISA TEST KIT FOR QUALITATIVE DETECTION OF *SALMONELLA* SPP.

Cat. Nos. 4323410301
4323410305

For 96 or 480 reactions

BAC*Spec*

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1 INTRODUCTION

The rapid detection of pathogens in foods is essential for ensuring the safety of consumers.

Traditional methods for detection of foodborne bacteria use time consuming growth in culture media followed by isolation, biochemical identification and serology.

ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) detection and identification is faster, more convenient and more specific than conventional methods.

This ELISA gives a presumptive result after a simple and efficient 37 h two step enrichment procedure.

The ELISA can be performed in less than two hours; the presumptive results can be obtained within 39 h or likewise on the third day after starting the sample in the pre-enrichment medium.

1.1 AOAC Performance Testing

The BAC*Spec Salmonella* 2 kit was reviewed by AOAC Research Institute and was found to perform to the manufacturer's specifications.

The kit is thus certified by AOAC-Research Institute under the Performance Tested Methods[SM] Program for detection of *Salmonella* spp. in:

25 g / 25 mL samples of:

- mayonnaise-based vegetable salad
- ground beef
- raw whole milk
- fresh spinach
- pasteurized liquid egg
- dry pet food

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 111903

- stainless steel environmental surface 1'' x 1'' (sampling with swabs)
- ceramic tile environmental surface 4'' x 4'' (sampling with sponges)

The end of the validity of the AOAC validation is indicated on the certificate 111903.

1.2 Intended Use

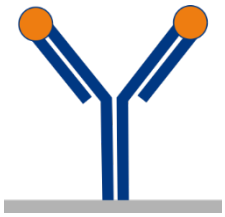
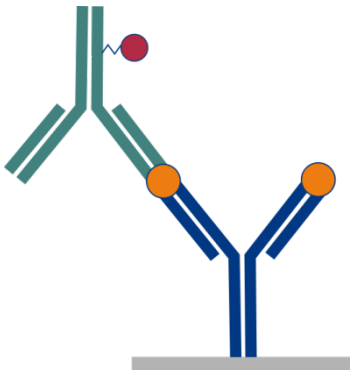
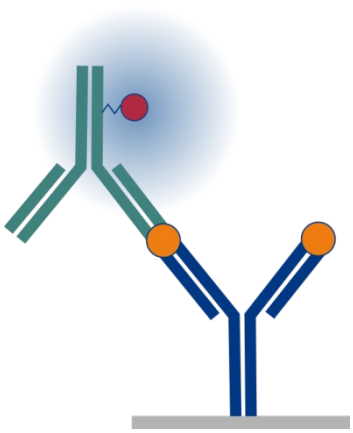
The BAC*Spec Salmonella* 2 ELISA kit provides materials for rapid in vitro detection of *Salmonella* spp. (including *S. enterica* and *S. bongori*) from human food products, feed products and environmental samples.

The BAC*Spec Salmonella* 2 ELISA kit is intended to be used in analytical laboratories for testing of human food products, feed products and environmental samples. It may also be applied for other purposes in food / feed product research and e.g. microbial monitoring of production processes.

The kit is not intended for clinical diagnostics and should therefore be regarded as "For Research Use Only".

1.3 Principle of the Assay

Food, feed and environmental samples are enriched in non-selective pre-enrichment buffer followed by secondary enrichment in a selective medium. An aliquot of the selective enriched sample is withdrawn and heat treated. After cooling, the samples are investigated for the presence of *Salmonella* antigens with a sandwich ELISA method.

Affinity purified antibody specific for *Salmonella* antigens is immobilized on the wells of microtiter strips.	
The heat treated samples, containing the bacterial antigens, are cooled to room temperature (18 – 27°C) and added to the antibody coated wells. *Salmonella* antigens present in the samples are bound immunologically by the antibody.	
After washing to remove unbound material, enzyme-conjugated affinity purified antibodies, which are specific for *Salmonella* antigens, are added to the wells. If *Salmonella* antigens are present in the samples, the enzyme-conjugated antibodies bind to the antigens and thus to the well.	
After a second washing step where any unbound enzyme-conjugated antibody is removed, enzyme substrate is added. A blue colour is formed by the action of bound enzyme on the substrate in those wells where *Salmonella* antigens are present. The reaction with the substrate is stopped after 30 min with diluted sulphuric acid, which changes the colour in the wells to yellow.	

1.4 Components of the Kit

The ELISA test kit contains 96 well microtiter plate(s), which can be separated into single strips of 8 wells. Each kit contains sufficient reagents for the microtiter plates included in the package. The kit expiration date is indicated on the label. Store the kit and kit components at 2 – 8°C protected from light. Do not freeze.

BAC*Spec Salmonella* 2 kit components (cat. nos. 4323410301 / 4323410305):

- 1x / 5x **ELISA plate(s)**, 96 / 480 wells, frame with 12 strips of 8 wells, coated with *Salmonella* antibodies, sealed into aluminium foil bag with desiccant pack
- 1x **Negative control**, 3 mL / 10 mL (colour coded green), contains stabilizer in working solution
- 1x **Positive control**, 3 mL / 10 mL (colour coded red), contains inactivated *Salmonella* Typhimurium with stabilizer in working solution
- 1x **Conjugate solution**, 12 mL / 60 mL (colour coded orange), contains horseradish peroxidase-antibody conjugate with stabilisers in working solution
- 1x **Substrate solution**, 12 mL / 60 mL (colour coded blue), contains 3,3',5,5'-tetramethylbenzidine (TMB) in working dilution (clear or faint blue solution). Avoid exposure to sun light and any other strong light
- 1x / 2x **Stop solution**, 12 mL / 30 mL (colour coded yellow), contains 0.2 M sulphuric acid
- 1x **Washing buffer concentrate**, contains 0.075 M Tris-HCl / 2.5 M NaCl with 5% Tween 20, pH 7.2. Diluted Washing Buffer may be stored for up to 1 week at room temperature protected from light
  - 6x 10 mL, dilute each vial 25 fold with 240 mL deionised or distilled water. 250 mL of diluted Washing Buffer is sufficient for 2 strips through both washing procedures of the test
  - 5x 60 mL, dilute each vial 25 fold with 1440 mL deionised or distilled water. 1.5 L buffer is enough to perform two full ELISA plates manually
- **Cover foil** for plate incubation

1.5 Additional Equipment, Consumables and Reagents Required

Equipment:

- 1x **Microtiter plate washer**
- 1x **Air incubator** for 37°C, accuracy ± 1°C
- 1x **Air incubator** for 37°C and 41.5°C, accuracy ± 1°C
- 1x **Micro-well plate reader** with 450 nm filter
- 1x **Stomacher** for homogenisation of samples
- 1x **Micro pipette** for transfer samples (0.1 mL) into the wells
- 1x **Multi-channel pipette** for transfer reagents (0.1 mL) into the wells
- 1x **Block heater / water bath** for 85 – 100°C, accuracy ± 5°C
- **Stopwatch**
- **Autoclave** for disinfection of samples
- **Automated ELISA analyser** (optional), which can fulfil the test conditions
  (Please contact customer service if automation is desired; BAC*Spec* Automation Protocol files for GSD Bolt and Thunderbolt are available on request).

Consumables:

- **Deionised or distilled water**
- **Tips for micropipette**
- **Flasks / stomacher bags / jars**, sterile, suitable for enrichment culture
- **Swabs / sponges / wipes** for environmental samples
- **Test tubes**, sterile (10 mL), suitable for selective enrichment culture
- **Transfer pipettes**, sterile (approx. 1 mL) for transfer of aliquots to tubes for heat treatment
- **Transfer pipettes**, sterile (approx. 0,1 mL) the pre-enriched sample to RVS Broth
- **Tubes**, heat resistant with caps
- **BPW, Buffered Peptone Water**, e.g. Biokar, cat. no. BM01008 or Oxoid, cat. no. CM0509
- **RVS, Rappaport-Vassiliadis Soya Peptone Broth,** e.g. Biokar cat. no. BK148HA
  or Oxoid, cat. no. CM0866

2 HOW TO USE THIS PRODUCT

Important Notes:

- Store all reagents as indicated in section 1.4.
- Do not use the reagents beyond the expiration dates printed on the labels.

2.1 Safety Precautions

- Warning: The Stop Solution contains sulphuric acid which is corrosive to metal, (Category 1), H290.
- All samples should be handled with caution as they are potentially infectious.
- All work should be performed by trained personnel in laboratories meeting Biosafety Level 2 (BSL2) regulations.
- The inactivated *Salmonella* Typhimurium in the positive control have been proven to be non-viable. The positive control should nonetheless be treated as potentially hazardous.
- Do not eat, drink or apply cosmetics in the work area where the test is performed.
- Do not pipette by mouth.
- Avoid contact of kit components with injured skin.

2.2 Working Guidelines

- Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).
- Reagents except the Washing Buffer 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.
- The kit should be stored at 2 – 8°C. Ensure that kit components are not exposed to temperatures higher than 40°C.
- Shelf life is indicated on the labels of the components.

2.3 Waste Disposal

- Dispose of any waste, which is potentially contaminated with pathogenic bacteria, according to your internal and local regulations.

2.4 Enrichment

For the preparation of test samples and initial suspensions follow the instructions of the ISO 6579, ISO 6887, ISO 7218 and ISO 18593 standards.

2.4.1 Preparation of the Enrichment Media

Ready-to-use media can be used for preparation of enrichment media.

If dried powder is used:

1. Dissolve Buffered Peptone Water (BPW) under magnetic stirring in distilled or deionised water.
    - Autoclave for 15 minutes at 121°C. Allow to cool to room temperature before applying to samples.
2. Dissolve Rappaport-Vassiliadis Soy broth (RVS) under magnetic stirring in distilled or deionised water.
    - Autoclave at 115°C for 15 minutes.
    - Dispense 10 mL into appropriate tubes (e.g. see section 1.5). Allow to cool to room temperature before applying sample.
3. Ensure an accuracy of 1% of the preparation.

All media should be at room temperature (18 – 27°C) before use.

Note: The quality of RVS broth varies from manufacturer to manufacturer. In order to avoid reduced assay performance, we advise to use Eurofins GeneScan Technologies' recommended RVS brands.

2.4.2 Pre-enrichment:

The pre-enrichment step for food, feed and environmental samples using BAC*Spec Salmonella* 2 is identical to ISO 6579-1:2017 as well as modifications for specific product types as outlined in ISO 6887-4:2017 and ISO 18593:2004.

Frozen food samples must be thawed at room temperature (18 - 27°C) before enrichment.

Protocols as performed for the AOAC PTM certification of BAC*Spec Salmonella* 2				
Sample Category	Protocol according to	Primary enrichment		
		Medium	Temp	Time
All human food products **25 g or mL**	ISO 6579-1:2017	1:10 BPW	37 ± 1°C	16 - 20 h
Feed products (incl. pet food) **25 g**				
Dusts, process water **25 g / mL**	ISO 18593:2004			
Swab **1**		10 mL BPW		
Wipe **1**		225 mL BPW		
Sponge **1**		100 mL BPW		

2.4.3 Selective Enrichment:

1. Transfer 0.1 ± 0.01 mL of the enriched sample to 10 mL of RVS broth.
2. Incubate for 21 – 27 hours at 41.5°C ± 1°C in an incubator or water bath.
3. Use pipettes and incubators of required accuracy.
4. Ensure that the bench time of RVS inoculated samples is kept to a minimum.
5. When processing large numbers of samples, transfer aliquot to tubes in individual racks and transfer single racks to incubator immediately. This is important to avoid extensive growth of competing microorganisms.
6. When the incubation period in RVS is completed, agitate the tubes gently and transfer a 0.5 – 1 mL aliquot to a sterile glass or heat resistant plastic test tube. Alternatively, store the RVS enrichment at 2 – 8°C for up to 72 hours before sampling.
7. After sampling, the un-heated enrichment samples should be kept at 2 – 8°C for verification until ELISA results are obtained.

2.5 Before you begin

8. Turn on heating block or water bath, and make sure that the temperature range of 85 – 100°C is reached.
9. Remove the test kit from cold storage (2 - 8°C) one hour before use to allow the components to warm to room temperature (18 – 27°C).
10. Dilute washing buffer concentrate 25-fold as described above.

2.6 Heat treatment of samples

1. Heat the aliquot to 85 – 100°C for 15 – 20 minutes in the closed test tube.
2. Cool the samples to room temperature (18 – 27°C).

2.7 ELISA Protocol

Please contact customer service if automation is desired; BAC*Spec* Automation Protocol files for GSD Bolt and Thunderbolt are available on request.

Note: Make sure samples are also at room temperature (18 – 27°C) before ELISA.

For manual performance, follow the next steps.

2.7.1 Transferring the Sample to ELISA Plate

1. Determine the number of wells required for the test. Take the required number of strips from the bag and fit them to the frame provided. Unused strips should be returned to the aluminium foil bag with the desiccant pack. Close the bag and store it at 2 - 8°C.
2. The first well (A1) in the first strip is reserved as a 'blank' for measuring the absorbance of the substrate and stop solution.
3. Pipette 0.1 mL of negative control into the second well (B1).
4. Pipette 0.1 mL of positive control into the third well (C1).
5. Pipette 0.1 mL of each 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.
6. Close the frame containing the strips with the cover foil and incubate for 30 minutes at 37°C ± 1°C. A prolongation up to 5 minutes is tolerated. Proceed immediately with the next step.
7. Wash the wells 5 - 7 times with diluted washing buffer. Preferably use a washing device. The washing technique is critical for the assay performance. Ensure complete filling and clearing of the wells through all steps of each washing cycle. At the end of the last cycle, remove as much liquid as possible.

2.7.2 Adding the Conjugate

8. Immediately after the washing procedure, pipette 0.1 mL of conjugate solution into all wells except the 'blank' (A1).
9. Close the frame with the cover foil and incubate for 30 minutes at 37°C ± 1°C. A prolongation up to 5 minutes is tolerated.
10. Wash the wells 5 - 7 times with diluted washing buffer as described above.

2.7.3 Adding the Substrate

11. Pipette 0.1 mL of substrate solution into all wells, including the ‘blank’ well (A1).
12. Incubate the wells with substrate for 30 minutes at room temperature (18 - 27°C; start the stop watch at well A1).

2.7.4 Stopping the reaction

13. Stop the colour reaction after 30 minutes by adding 0.1 mL of stop solution to all wells including the ‘blank’ well. The stop solution will cause the blue colour to change to yellow in wells with positive samples.
14. Read optical densities (OD) within 20 minutes in a microtiter plate reader with a 450 nm filter. The photometer should be zeroed against the ‘blank’ well (usually A1) before the other wells are read. Alternatively, the blank value will need to be subtracted from the measured value for each sample manually during the evaluation.

If the user is familiar with the procedure, the “Short Instructions” description on the back of this booklet may be followed.

3 DATA INTERPRETATION

The absorptions, expressed as optical densities (OD), are measured with a microtiter plate reader. The background (OD value of blank well, e.g. A1) is subtracted as described in 2.8.4 and the result compared to the cut-off value to determine the presence of *Salmonella*.

The OD reading of the negative control must be below 0.250 and the OD reading of the positive control must be above 0.500.

The cut-off for a positive call using the BAC*Spec Salmonella* 2 kit is defined as follows:

$$\text{Cut-off} = OD_{pos} \times 25\%$$

With OD_{pos}: OD value (blanked) of positive control.

Samples from wells with OD values (blanked) above the cut-off are considered positive for *Salmonella*.

4 CONFIRMATION OF PRESUMPTIVE POSITIVE RESULTS

All samples identified as positive by the BAC*Spec Salmonella* 2 ELISA kit must be confirmed by one of the following test(s):

Option 1:

Using the conventional tests described in the method standardised by ISO from colonies (including the purification step).

Option 2:

Streaking 10µL of RVS enrichment onto Xylose-Lysin-Desoxycholat-Agar (XLD) and an additional chromogenic agar followed by biochemical galleries directly on isolated typical colonies without a purification step.

Independent of the confirmation method used, in the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above, in particular by the serological test) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

5 PERFORMANCE LIMITATIONS AND RECOMMENDATIONS FOR USE

Proper performance of the enrichment phase is important for the assay. Use Eurofins GeneScan Technologies' recommended media for optimal performance. Other media may cause reduced performance of the kit. Test performance has been optimised using the culture reagents specified above and it is strongly recommended that only these media be used.

Ensure correct temperature setting of the incubator used for selective enrichment using a thermometer calibrated to a certified standard.

Washing of the wells is a critical step. Do not attempt to use squeeze bottles or other uncontrolled devices.

Use multichannel pipettes or preferably multichannel washing devices designed for micro-plates. Automatic devices give optimal results.

The enzyme peroxidase used in the kit will be inactivated in the presence of sodium azide. Therefore ensure that residues of sodium azide are not present in washing devices, buffer reservoirs, tubes or the immuno-washer.

6 PROTOCOL AT A GLANCE

Enrichment Protocol

	Enrichment Procedure	Recommendations
STEP A	Homogenise 25 g sample by stomaching in 225 mL BPW	BPW at room temperature (18 – 27°C) before use. Pre-enrichment according to ISO 6579-1 standard.
	Incubate for 16 – 20 hours at 37°C ± 1°C.	
STEP B	Transfer 0.1 mL to 10 mL RVS	Do not over-inoculate. Use RVS recommended by Eurofins GeneScan Technologies. Limit handling time of RVS inoculated samples. Higher numbers of samples should be split and each portion should be transferred to the incubator/water-bath without delay.
	Incubate for 21 – 27 hours at 41.5°C ± 1°C	Use water-bath with circulation or accurate incubator with homogenous temperature distribution.
STEP C	Re-suspend samples and transfer 0.5 - 1 mL to glass or polypropylene vials. Use closures or heat resistant foil to cover the vials. Heat at 85 – 100°C for 15 – 20 minutes. Cool to room temperature prior to ELISA.	Always keep the heat treated RVS samples until the ELISA result is approved. Note: Heat treated RVS samples may be stored at 2 – 8°C for 2 weeks (not within the scope of certifications).

ELISA Protocol

STEP 1	Well A1 is reserved as blank control 0.1 mL negative control into well B1 0.1 mL positive control into well C1 0.1 mL heat treated and cooled sample per well
Incubation:	30 minutes at 37°C ± 1°C (a prolongation of up to 5 minutes is tolerated)
Washing:	Wash the wells with diluted washing buffer

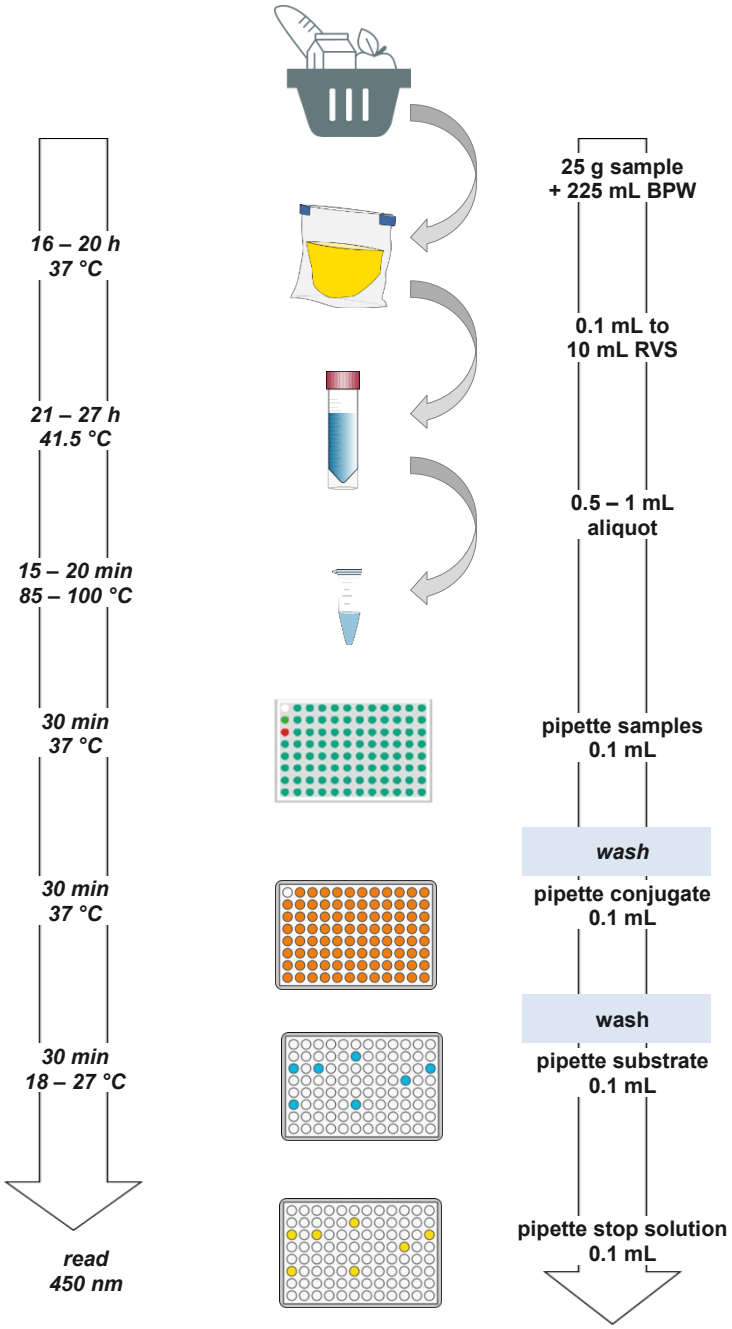
STEP 2	0.1 mL conjugate into all wells except blank (A1)
Incubation:	30 minutes at 37°C ± 1°C (a prolongation of up to 5 minutes is tolerated)
Washing:	Wash the wells with diluted washing buffer

STEP 3	0.1 mL substrate into all wells including blank (A1)
Incubation:	30 minutes at room temperature (18 – 27°C) (a prolongation of up to 1 minute is tolerated)

STEP 4	0.1 mL stop solution into all wells including blank (A1)
Read:	Read within 20 minutes at 450 nm

BAC*Spec Salmonella* 2

SHORT INSTRUCTIONS



7 PRODUCT WARRANTIES, SATISFACTION GUARANTEE

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8 IMPORTANT NOTES

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TECHNICAL SUPPORT SERVICE

For technical assistance and more information please contact the Eurofins GeneScan Technologies GmbH Customer Service or your local distributor.

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