



# NordVal International Certificate

Issued for:	<b>foodproof<sup>®</sup> <i>Salmonella</i> Detection Kit, Hybridization Probes and foodproof<sup>®</sup> <i>Salmonella</i> Detection Kit, 5' Nuclease, in combination with foodproof<sup>®</sup> ShortPrep I Kit or foodproof<sup>®</sup> StarPrep One Kit</b>
NordVal No:	023
First approval date:	28 October 2005
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Valid until:	10 June 2019

**foodproof<sup>®</sup> *Salmonella* Detection Kit, Hybridization Probes and foodproof<sup>®</sup> *Salmonella* Detection Kit 5' Nuclease in combination with foodproof<sup>®</sup> ShortPrep I Kit or foodproof<sup>®</sup> StarPrep One Kit**

Manufactured and supplied by:  
BIOTECON Diagnostics GmbH,  
Hermannswerder 17,  
14473 Potsdam, Germany.

fulfils the requirements of the NordVal validation protocol. The reference method was EN ISO 6579:2002 for detection of *Salmonella* spp. in food, animal feed and environmental samples.

NordVal International has reviewed the method and the validation studies conducted by the MQD, Institute for Analytic and Hygiene in Güstrow, Germany, studied the enclosures to the application and evaluated the results obtained in the validations. The results document no statistical differences in the performances between the alternative method and the reference method. NordVal International has concluded that it has been satisfactorily demonstrated that the requirements for the sensitivity and the agreement between the methods are fulfilled, further, that confirmation of obtained positives are not necessary.

Date: 9 June 2017

Yours sincerely

A handwritten signature in blue ink, appearing to read 'Hilde Skår Norli'.

Hilde Skår Norli  
Chair of NordVal International

A handwritten signature in blue ink, appearing to read 'Nina Skall Nielsen'.

Nina Skall Nielsen  
NMKL Secretary General



## PRINCIPLE OF THE METHOD

The principle is real-time PCR and detection with specific, fluorescence labelled probes.

After DNA isolation using the **foodproof**<sup>®</sup> ShortPrep I Kit (Art. No. S 400 01), or the bulk version of this kit, the **foodproof**<sup>®</sup> StarPrep One Kit (Art. No. S 400 07) both designed for the rapid preparation of bacterial DNA for direct use in PCR, the real-time detection of *Salmonella* DNA is carried out either by using the **foodproof**<sup>®</sup> *Salmonella* Detection Kit, Hybridization Probes (Art. No. 300 27) or the **foodproof**<sup>®</sup> *Salmonella* Detection Kit, 5'Nuclease (Art. No. R 302 27).

For food and feed samples inoculate 25 g. For environmental samples inoculate an area of 100 cm<sup>2</sup>. Perform the pre-enrichment according to EN ISO 6579. The detection kit provides all the reagents required for the PCR.

## FIELD OF APPLICATION

The **foodproof**<sup>®</sup> *Salmonella* Detection Kit, Hybridization Probes and the **foodproof**<sup>®</sup> *Salmonella* Detection, 5'Nuclease in combination with **foodproof**<sup>®</sup> ShortPrep I Kit or the **foodproof**<sup>®</sup> StarPrep One Kit for the detection of *Salmonella* are intended for the detection of *Salmonella* DNA isolated. The methods are tested on foods, feeds and environmental samples.

## HISTORY

The **foodproof**<sup>®</sup> *Salmonella* Detection Kit, Hybridization in combination with **foodproof**<sup>®</sup> ShortPrep I Kit was first approved in 2005 based on a comparison study and a collaborative study.

In 2011, the method was extended: A new system was evolved using hydrolysis probes instead of hybridisation probes. The modification, using a new primer, required a new comparison study of the selectivity (inclusivity and exclusivity) and a comparison study of the relative accuracy to measure the degree of correspondence between the results obtained by the **foodproof**<sup>®</sup> *Salmonella* Detection kit and the reference method. As the method procedure was unchanged, NordVal did not require an additional collaborative study.

In 2017, the results obtained for the **foodproof**<sup>®</sup> *Salmonella* Detection Kit, Hybridization Probes and **foodproof**<sup>®</sup> *Salmonella* Detection Kit 5' Nuclease in combination with **foodproof**<sup>®</sup> ShortPrep I Kit or **foodproof**<sup>®</sup> StarPrep One Kit method have been recalculated according to the ISO 16140-2:2016 protocol.

## METHOD PERFORMANCE CHARACTERISTICS

### Selectivity: Inclusivity/exclusivity

The relative selective was satisfactory in the study approved in 2005.

The additional study approved in 2011:

Inclusivity: All the 54 isolates from *Salmonella* spp. were positively detected.

Exclusivity: All the 30 bacteria from *Enterobacteriaceae* and other closely related bacteria or organisms of the same habitat were negative.

The selectivity, i.e. the inclusivity and exclusivity, was 100%.



### Relative accuracy, relative sensitivity and relative specificity:

The results of the study carried out in 2005 were satisfactory.

The results of study approved in 2011:

Three different sub matrices of meat (minced meat, poultry meat, salami), feed (fish feed, bone meal, chew sticks for dogs) and environmental samples (metal surface, glass surface, plastic surface), respectively, were included in the study. Two strains relevant for each matrix were selected. Three inoculation levels were used: 0 = negative control, 1-10 cells per 25 g / area of 100 cm<sup>2</sup> sample and 10-100 cells per 25 g / area of 100 cm<sup>2</sup> sample. For each matrix 60 samples were analysed.

The following results were obtained:

Table 1: Results after screening

Matrix	* PA	NA	ND	PD	Sum	Relative AC %	Relative SE %	Relative SP %	Kappa
Meat	39	21	0	0	60	100	100	100	1.0
Environmental	21	39	0	0	60	100	100	100	1.0
Feed	32	28	0	0	60	100	100	100	1.0
Total	92	88	0	0	180	100	100	100	1.0

PA = number of obtained results that are positive with both the alternative and the reference method

NA = number of obtained results that are negative with both the alternative and the reference method.

ND = number of obtained results that are negative with the alternative method and positive with the reference method (possible false negative)

PD = number of obtained results that are positive with the alternative method and negative with the reference method (possible false positive)

Relative AC = The relative accuracy; the degree of correspondence between the response obtained by the alternative method and the reference method.

Relative SE = The relative sensitivity; the ability of the alternative method to detect the analyte compared to the reference method

Relative SP = The relative specificity is the ability of the alternative method not to detect the target microorganism when it is not detected by the reference method.

Kappa = The degree of agreement between the alternative method and the reference method, kappa of 0,80 or higher is considered to be very good agreement.

It was confirmed that the results obtain did not contain any false positives nor false negatives, and hence the Acceptability Limits for the sensitivity according to ISO 16140-2, were met. The alternative method and the reference method provide equivalent results.

### Detection Level

The limit of detection is 1-10 cells per 25 g, which was obtained both with the alternative method and the reference method for all matrices.

### CONCLUSION

The **foodproof**<sup>®</sup> *Salmonella* Detection Kit, Hybridization Probes and the **foodproof**<sup>®</sup> *Salmonella* Detection, 5'Nuclease in combination with **foodproof**<sup>®</sup> ShortPrep I Kit or the **foodproof**<sup>®</sup> StarPrep One Kit for the detection of *Salmonella* perform equivalent to the reference method.