



foodproof® *Enterobacteriaceae* plus *Salmonella* Detection LyoKit

Revision A, January 2024

PCR kit for the qualitative detection of *Enterobacteriaceae* plus simultaneous identification of *Salmonella* spp. DNA using real-time PCR instruments.

Product No. KIT230137 (LP)

KIT230138 (RP)

KIT230139 (DP)

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store the kit at 2 to 8 °C

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FOR *IN VITRO* USE ONLY



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1. What This Product Does

1.1 Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µL each. Up to 94 samples (single sample preparation) plus positive control template and negative control reactions can be analyzed per run.

1.2 Storage and Stability

- Store the kit at 2 to 8 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Contents table:

1.3 Kit Contents

Component	Label	Contents / Function / Storage
foodproof® <i>Enterobacteriaceae</i> plus <i>Salmonella</i> Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat <ul style="list-style-type: none"> • KIT230140 with white low-profile tubes (LP) • KIT230141 with clear regular-profile tubes (RP) • KIT230142 with clear low-profile tubes (DP) 	<ul style="list-style-type: none"> • 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for DNA of <i>Salmonella</i>, <i>Enterobacteriaceae</i> and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil-DNA N-Glycosylase (UNG, heat-labile) for prevention of carry-over contamination. • Store at 2 to 8 °C in the aluminum bag (sealed). • Protect from light and moisture!
Positive Control	Vial 2 (purple cap)	<ul style="list-style-type: none"> • 1 x 300 µL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
Negative Control	Vial 3 (colorless cap)	<ul style="list-style-type: none"> • 2 x 1 mL • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	<ul style="list-style-type: none"> • 12 x 8-cap strip • For use in real-time PCR after addition of samples.

1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM, VIC/HEX, and ROX labeled probes as well as for using low or regular profile strip tubes. In cases the strip tubes don't fit for the instrument the samples have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- For users of the LightCycler® 480 II (Roche Diagnostics) a color compensation kit (Color Compensation Set 5; Product No. KIT230011) and a special adapter for PCR strips are necessary. Please contact [Hygiena Diagnostics](#) for further information.



- Sample Preparation Kits:
 - foodproof StarPrep One Kit (Product No. KIT230175/ 76) or
 - foodproof StarPrep One 8-Strip Kit (Product No. KIT230183) or
 - foodproof StarPrep Three Kit (Product No. KIT230187) or
 - foodproof StarPrep Three 8-Strip Kit (Product No. KIT230188)
 - foodproof Reagent D (Product No. KIT230001/ 02/ 03)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes

and optionally:

- Vortex centrifuge Multispin MSC-6000 for PCR-strips with SR-32, Rotor for MSC-6000 or
- Vortex centrifuge CVP-2 for PCR-plates

1.5 Applicability Statement

The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit is intended for the rapid detection of *Enterobacteriaceae*, including simultaneous identification of *Salmonella* spp. DNA isolated from enrichment cultures with enrichment conditions (e.g., recommended in the ISO methods for *Salmonella* (ISO 6579-1:2017)) by using the above-mentioned sample prep methods for all relevant kinds of foods, feeds and environmental samples that are potentially contaminated with *Salmonella* or other *Enterobacteriaceae*. The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit is destined for the food and feed industry and for food testing laboratories.

The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM, a VIC/Yakima Yellow or HEX and a ROX or Texas Red detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler[®] 480 II, LightCycler[®] 96 (Roche Diagnostics), Mx3005P[®], AriaMx (Agilent Technologies), ABI 7500[®] Fast (Thermo Fisher), CFX96[™] (Bio-Rad), Dualo 32[®] R².

The performance of the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit in combination with the foodproof StarPrep One Kit and the foodproof StarPrep Three Kit in both single tube and 8-strip formats has been approved in a MicroVal[®] method validation according to ISO 16140-2:2016 (Certificate No. 2020LR90). For this validation study the following categories were tested: infant cereals, infant formula with or without probiotics and ingredients and production environmental samples. For further information about the enrichment protocols please refer to ANNEX 1 at the end of the package insert.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of *Enterobacteriaceae* and *Salmonella* DNA using the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit requires DNA amplification by PCR. The kit provides all reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carry-over-, or cross-contamination:

- **Keep the kit components separate** from other reagents in the laboratory.
- Use **nuclease-free labware** (e.g., pipettes, pipette tips, reaction vials).



- Wear gloves when performing the assay.
- To **avoid cross-contamination** of samples and reagents, use fresh **aerosol barrier pipette tips**.
- To **avoid carry-over contamination**, transfer the required solutions for one experiment into a **fresh tube**, rather than directly pipetting from stock solutions.
- Physically **separate the workplaces** for DNA preparation, PCR setup, and PCR to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof *Enterobacteriaceae* plus *Salmonella* lyophilized PCR Mix away from light and moisture.

2.1.2 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from various samples, refer to the corresponding product package inserts of a suitable sample preparation kit (see “*Additional Equipment and Reagents Required*”).

2.1.3 DNA Extraction

Hygiena Diagnostics provides sample preparation kits suitable for all kinds of food samples and PPS (see “*Additional Equipment and Reagents Required*”).

For more product information, please refer to www.hygiena.com.

2.1.4 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof *Enterobacteriaceae* plus *Salmonella* Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.5 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with foodproof *Enterobacteriaceae* plus *Salmonella* Negative Control (vial 3, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.1.6 Cultural Confirmation

Positive PCR results can be confirmed with cultural confirmation methods, e.g., recommended by the reference methods for *Salmonella* (ISO 6579-1:2017) or *Enterobacteriaceae* (ISO 21528-1:2017). For further information, please visit the following web address: www.iso.org.

2.2 Procedure

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (*Salmonella*), VIC/HEX (*Enterobacteriaceae*) and ROX (Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Operator’s Manual of your real-time PCR cycler:



<u>Pre-incubation</u>	1 cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
<u>Amplification</u>	45 cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 30 seconds
Step 3:	72 °C for 60 seconds

*Fluorescence detection in step 2

Notes:

For some real-time PCR instruments, the type of probe quencher as well as the usage of a passive reference dye has to be specified. The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit contains probes with a non-fluorescent (“dark”) quencher and no passive reference dye.

For users of the Agilent Mx3005P instrument: Click ‘Instrument → Filter Set Gain Settings’ to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. For FAM, HEX and ROX, the Filter Set Gain Setting has to be modified to ‘x1’.

2.2.2 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction. Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

Note: PCR strips must be stored in the provided aluminum bag with silica gel pads to avoid liquid absorption.

Note: The lyophilizate is only stable in the provided aluminum bag with the silica gel pad.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterward and store under the recommended conditions.
2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.
3. Uncap the tube strips cautiously and discard the cap strips.

Note: To avoid unwanted liquid absorption, open strips immediately before filling.

4. Pipet 25 µL sample into each PCR vessel:
 - For the samples of interest, add 25 µL sample DNA (if less, then add PCR-grade H₂O to achieve 25 µL).
 - For the negative control, add 25 µL foodproof *Enterobacteriaceae* plus *Salmonella* Negative Control (vial 3, colorless cap).
 - For the positive control, add 25 µL foodproof *Enterobacteriaceae* plus *Salmonella* Positive Control Template (vial 2, purple cap).

Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.

5. Seal the vessels accurately and tightly with the colorless cap strips.



- Mix thoroughly using a vortex centrifuge.

Note: Hygiena Diagnostics recommends vortex centrifuges Multispin MSC-6000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for these centrifuges. Alternatively, resuspend the pellet by manually mixing by cautiously pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down on the strip caps.

- Spin the PCR tube strips for 30 seconds at 150–200 x g in a suitable centrifuge. If your centrifuge exceeds 200 x g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1,000 x g!
- Place the samples in your PCR cycler and run the program as described above.

Note: For some PCR instruments, the PCR strips should be placed in a special adapter and a balanced order in the cycler block. For example, two strips can be placed in columns 1 and 12 (see “Additional Equipment and Reagents Required”).

2.3 Data Interpretation

2.3.1 Procedure – Qualitative Detection

For qualitative detection, compare the results from channels FAM (*Salmonella*), VIC/HEX (*Enterobacteriaceae*), and ROX (Internal Control) for each sample and interpret the results as described in the table below.

Channel FAM	Channel VIC/HEX	Channel ROX	Result Interpretation
Positive	Positive	Positive or Negative	Positive for <i>Enterobacteriaceae</i> and <i>Salmonella</i>
Negative	Positive (Cq < 40)	Positive or Negative	Positive for <i>Enterobacteriaceae</i> (non- <i>Salmonella</i>)
Negative	Negative (Cq ≥ 40)	Positive	Negative for <i>Enterobacteriaceae</i> and <i>Salmonella</i>
Negative	Negative (Cq ≥ 40)	Negative	Invalid

If the amplification in channel HEX is very weak (Cq > 35), the result in FAM may be negative due to slight differences in the assays’ limit of detection in this multiplex PCR system. In this case, a second enrichment and a repetition of the analysis is recommended.

For the data analysis at LightCycler 480 II instrument, it is recommended to use the ‘Abs Quant/Fit Points’ Analysis Type or the microproof Diagnostic Interpreter Tool and a color compensation set (Color Compensation Set 5; Product No. KIT230011).

For questions on the diagnostic interpreter tool or other PCR instruments (as mentioned above in “Applicability Statement”), please contact Hygiena Diagnostics Technical Support (www.hygiena.com/support) for assistance.

Note: A prerequisite for the unambiguous discrimination of the targets in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, HEX, and ROX. Please refer to the operation manual of your real-time PCR cycler for further information.



3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	<ul style="list-style-type: none"> Set Channel settings to FAM, HEX, ROX
	Pipetting errors.	<ul style="list-style-type: none"> Check for correct reaction setup. Repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	<ul style="list-style-type: none"> Check the cycle programs.
No signal increase in channel ROX is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> Use the recommended DNA sample preparation kit to purify template DNA. Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µL instead of 25 µL).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> Store the foodproof <i>Enterobacteriaceae</i> plus <i>Salmonella</i> Detection lyophilized PCR mix at 2 °C to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	<ul style="list-style-type: none"> Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline	Resuspension of lyophilized PCR mix not complete	<ul style="list-style-type: none"> Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> Exchange all critical solutions. Repeat the complete experiment with fresh aliquots of all reagents. Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.	<ul style="list-style-type: none"> Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	<ul style="list-style-type: none"> Always wear gloves when handling the vessels and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	<ul style="list-style-type: none"> Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad. Open strip immediately before filling.



4. Additional Information on this Product

4.1 How this Product Works

The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the ROX channel, whereas the *Salmonella* DNA is detected in channel FAM and the *Enterobacteriaceae* DNA in the HEX channel. In case of a negative result due to inhibition of the amplification by the sample DNA of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of *Enterobacteriaceae* and *Salmonella* in the sample. The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of *Enterobacteriaceae* DNA. Primers and probes provide specific detection of *Enterobacteriaceae* and *Salmonella* DNA in food samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

4.2 Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of specific sequences for the target *Enterobacteriaceae* and *Salmonella* species.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5' nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5'-end with a reporter fluorophore and at the 3' end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal amplicon sequence and is cleaved by the 5' nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

4.3 Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated *Enterobacteriaceae* genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit, decontamination can be achieved with the provided reagents.

4.4 Product Characteristics

4.4.1 Specificity:

In-/exclusivity of the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit has been tested with 72 strains comprising of the family *Enterobacteriaceae* (equal to new order *Enterobacteriales*) plus 38 strains of the genus *Salmonella* and as well as more than 50 non-*Enterobacteriaceae* (new non-*Enterobacteriales*) species (mostly of the closely related genera) [1,2]. All *Salmonella* strains were detected in FAM and HEX/VIC channels, all



non-*Salmonella Enterobacteriaceae* in channel HEX/VIC and none of the non-*Enterobacteriaceae* strains were detected in any channel.

4.4.2 Sensitivity:

A relative detection limit of 1 to 10 cells per 25/100 g sample can be achieved with all relevant kinds of foods. The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit detects down to 10^2 - 10^3 CFU/mL of *Enterobacteriaceae* / *Salmonella* enrichment culture (depending on the sample preparation kit used).

4.4.3 Temperature robustness:

The temperature limits of the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit are the following:

Denaturation temperature: 95 ± 1.75 °C / -2.75 °C

Annealing temperature: 60 °C ± 2.75 °C

The limits were determined according to Annex C of ISO/DIS 20836:2020.

Real-time thermal cyclers are compatible with the real-time PCR assay when operating within the stated temperature specification limits.

4.5 References

1. Ewing WH, Farmer Iii JJ, Brenner DJ. "Proposal of Enterobacteriaceae fam. nov., nom. rev. to replace Enterobacteriaceae Rahn 1937, nom. fam. cons. (Opin. 15, Jud. Comm. 1958), which lost standing in nomenclature on 1 January 1980". *Int. J. Syst. Bacteriol.* 1980; 30:674-675.
2. Adeolu M., Alnajjar S., Naushad S., Gupta R.S. "Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov.". *Int. J. Syst. Evol. Microbiol.* 2016; 66(12):5575-5599.

4.6 Quality Control

The foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit is function tested using the LightCycler® 480 II System.



5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview of our products and for more information, please visit our website at www.hygiena.com.

5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under US Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008.

Email: outlicensing@lifetech.com.

5.3 Trademarks

foodproof[®] is a registered trademark of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 602 67



5.6 ANNEX 1: Validation Information on the MicroVal Study according to ISO 16140-2:2016 for the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit, Certificate No. 2020LR90

The following table shows the enrichment procedure in Buffered Peptone Water (BPW) for the food categories and production environmental samples that have been analyzed for the MicroVal method validation of the foodproof *Enterobacteriaceae* plus *Salmonella* Detection LyoKit in combination with foodproof StarPrep One Kit and foodproof StarPrep Three Kit (single tube and 8-strip).

For further information regarding the DNA extraction procedures below, please refer to the appropriate Hygiena Diagnostics package inserts on www.hygiena.com.

DNA Extraction	Enrichment Time in BPW ^{1,2,3} at 37 ± 1 °C	DNA Extract for PCR
foodproof StarPrep One Kit (Product No. KIT230175/ 76) Extraction Procedure B: LIVE/DEAD	18 ± 2 h	25 µL
foodproof StarPrep One 8-Strip Kit (Product No. KIT230183) Extraction Procedure D: LIVE/DEAD	18 ± 2 h	25 µL
foodproof StarPrep Three Kit (Product No. KIT230187) Manual: StarPrep Three Kit for <i>Enterobacteriaceae</i> plus <i>Salmonella</i> Extraction Procedure A: STANDARD (LIVE/DEAD)	18 ± 2 h	25 µL
foodproof StarPrep Three 8-Strip Kit (Product No. KIT230188) Manual: StarPrep Three Kit for <i>Enterobacteriaceae</i> plus <i>Salmonella</i> Extraction Procedure B: HIGH THROUGHPUT (LIVE/DEAD)	18 ± 2 h	25 µL

Tested Categories & Sample Sizes:

1. Infant cereals, infant formula with or without probiotics and ingredients
 - 375 g + 3375 mL pre-warmed Buffered Peptone Water^{1,2,3}
2. Production environmental samples
 - 200 g + 1800 mL pre-warmed Buffered Peptone Water
 - 1 swab + 10 mL Buffered Peptone Water
 - 1 sponge + 100 mL Buffered Peptone Water
 - 1 wipe + 225 mL Buffered Peptone Water
 - For sampling after cleaning processes, premoisten:
 - 1 swab + 1 mL broth, universal neutralizing (+ 9 mL Buffered Peptone Water)
 - 1 sponge + 10 mL broth, universal neutralizing (+ 90 mL Buffered Peptone Water)
 - 1 wipe + BPW + 10 % neutralizing agent (+ 225 mL Buffered Peptone Water)

Reference Methods: ISO 21528-1 (2017), ISO 6579-1 (2017), ISO 6579-1/A1 (2020)

¹Use BPW when testing infant formula or infant cereals with *Lactobacillus reuteri*, *Lactobacillus paracasei*, *Lactobacillus fermentum*.

²The Addition of alpha-amylase (0.1 g/L) is required for cereal-containing products.

³The Addition of vancomycin (10 mg/L) is required for products containing the probiotic *Bifidobacterium lactis*.

If a matrix is to be tested that contains probiotics but not tested within this study, vancomycin should be added during enrichment.



6. Change Index

Version 1, March 2020

First version of the package insert.

Version 2, December 2020

Specifications on data analysis.

Version 3, May 2022

Addition of MicroVal logo.

Additional information in the Applicability Statement.

Addition of ANNEX 1.

Revision A, January 2024:

Rebranding and new layout.

R 602 67 20 -> INS-KIT230137-38-39-RevA



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