

FOR *IN VITRO* USE ONLY

foodproof[®] STEC Screening LyoKit – 5'Nuclease –

Version 3, October 2020

PCR kit for the qualitative detection of Shiga toxin-producing *Escherichia coli* (STEC) by screening for genes of Shiga toxins (*stx1* & *stx2*) and intimin (*eae*) using real-time PCR instruments.

Order No.: R 602 11-1 / R 602 11-2

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

Store the kit at 2 °C to 8 °C



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1. What this Product Does

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 and negative control reactions can be analyzed per run.

Storage and Stability

- Store the kit at 2 °C 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:

Kit Contents

Component	Label	Contents / Function / Storage
foodproof® STEC Screening LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing a 8-tube strip mat • R 602 11-1 with white low profile tubes* • R 602 11-2 with clear regular profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for the virulence genes <i>stx1</i> -, <i>stx2</i> -, <i>eae</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. • For amplification and detection of STEC-specific sequences. • Store at 2 °C to 8 °C in the aluminium bag (sealed). • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	• 1 x 250 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 °C to 8 °C.
H ₂ O PCR-grade	Vial 3 (colorless cap)	• 2 x 1 ml • Nuclease-free, PCR-grade H ₂ O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples.

*Tube profile and instrument compatibility chart is available online: www.bc-diagnostics.com/compatibility-chart

Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes as well as for using low or regular profile strip tubes. In cases the strip tubes do not fit the PCR instrument, the samples will have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- Sample Preparation Kit
 foodproof® StarPrep Three Kit (Order No.: S 400 18)¹ or
 foodproof® Magnetic Preparation Kit I (Order No. S 400 11)¹
- Filter pipette tips. Nuclease free, aerosol resistant
- Microliter Pipettes
- Vortex centrifuge Multispin MSC-6000 for PCR strips (Order No.: D 110 66)¹ **with** SR-32, Rotor for MSC-3000/6000 (Order No.: D 110 65)¹ **or**
- Vortex centrifuge CVP-2 for PCR plates (Order No.: D 110 67)¹

¹ Available from BIOTECON Diagnostics; see ordering Information for details

Applicability Statement

The **foodproof® STEC Screening LyoKit – 5'Nuclease** – is intended for the rapid detection of Shiga toxin-producing *E. coli* DNA isolated from enrichment cultures prepared by valid methods and inoculated with all relevant kinds of foods and primary production stage (PPS) samples that are potentially contaminated with Shiga toxin-producing *E. coli*.

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 HEX, a ROX and a Cy5 detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), AriaMx, Mx3005P® (Agilent Technologies), CFX96 (Bio-Rad), ABI 7500 fast (Applied Biosystems) and PikoReal® 24 (Thermo Scientific).

Note: A Color Compensation (Color Compensation Set 3; Order No. A 500 10) is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 Systems I and II. Please contact BIOTECON Diagnostics for further information.

The **foodproof®** STEC Screening LyoKit was validated according to the AOAC RI PTM program (license number 102004) for the category raw meat for screening and for confirmation of Shiga toxin- (*stx1* & *stx2*) and intimin- (*eae*) positive *E. coli* STEC strains.

The validation includes 375 g test portions enriched in mTSB (1:4) at 42 ± 1 °C for 12-24 h and 25 g test portions enriched in mTSB (1:10) for 8-24 h. DNA isolation was done according to the package insert of the **foodproof®** StarPrep Three Kit – Extraction Procedure A. For 25 g meat samples with 8-20 h enrichment time and for 375 g meat samples with 12-20 h enrichment time, 500 µl of the enrichment culture were used for DNA extraction. For enrichment times between 20 h and 24 h 100 µl of the enrichment culture were used for DNA extraction. The **foodproof®** STEC Screening LyoKit was validated in combination with the **foodproof®** STEC Identification LyoKit and positive samples were confirmed via the method described in Annex A in the package insert of the **foodproof®** STEC Identification LyoKit and via the reference method described in the USDA/FSIS-MLG 5C.00.

2. How to Use this Product

2.1 Before You Begin

Safety and Precautions

Detection of STEC DNA using the **foodproof®** STEC Screening 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-preventive 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.

Follow all universal safety precautions governing work with biohazardous materials, e.g. wear lab coats, gloves and other personal protective equipment at all times. The assay should only be used by adequately trained personnel. Properly dispose of all contaminated materials, clean and decontaminate work surfaces with an appropriate disinfectant of choice (e.g., sodium hypochlorite solution) before and after use as part of aseptic techniques. Use a biosafety cabinet whenever aerosols might be generated. In addition to cleaning workstations, work areas should be separated for the following: media preparation, sample preparation, and pathogen detection. Laboratory equipment like pipettes or tubes must not circulate between workstations. When working with enrichment cultures, filter laboratory bags should be used to minimize particulates and shaking the enrichment bag or collecting large food fragments should be avoided. For fatty foods, collect the sample just below the fat layer. Never reuse kit disposables and always change serological pipettes and pipette tips between samples.



Waste Disposal

All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For more information, e.g. proper disposal of unused chemicals, please refer to the appropriate safety data sheet (SDS). The SDS is available online at www.bc-diagnostics.com.

Keep the foodproof® STEC Screening lyophilized PCR Mix away from light and moisture.

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 sample enrichments, refer to the corresponding product package inserts of a suitable sample preparation kit (see “*Additional Equipment and Reagents Required*”).

DNA Extraction

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

For more product information please refer to www.bc-diagnostics.com.

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®** STEC Screening Control Template (vial 2, purple cap)] or with a positive sample preparation control.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H₂O PCR-grade (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.

Cultural Confirmation

If the kit is used in combination with the **foodproof®** STEC Identification LyoKit and a confirmation step is required, conventional tests described in the methods standardized by ISO, USDA or FDA from colonies (including the purification step) can be used. Alternatively the **foodproof®** STEC confirmation method described in Annex A in the package insert of the **foodproof®** STEC Identification LyoKit can be used.



2.2 Procedure

Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (for *stx1* gene), HEX (for *stx2* gene), ROX (for *eae* gene) and Cy5 (for Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the **foodproof**[®] STEC Screening LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR cycler:

<u>Preincubation</u>	1 cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
<u>Amplification</u>	50 cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection in step 2

Notes:

- For some real-time PCR instruments the type of the probe quencher as well as the usage of a passive reference dye has to be specified. The **foodproof**[®] STEC Screening 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 the Filter Set Gain Setting has to be modified to "x1".



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

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away at 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. Decap the tube strips cautiously and discard the cap strips.

Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips only shortly before filling.

4. Pipet 25 µl sample into each PCR vessel:

- For the samples of interest, add 25 µl sample DNA (if using less volume, add PCR-grade H₂O to achieve 25 µl).
- For the negative control, add 25 µl PCR-grade H₂O (vial 3, colorless cap).
- For the positive control, add 25 µl **foodproof**[®] STEC Screening 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.
6. Mix thoroughly using a vortex centrifuge.

Note: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-3000 (Order No.: D 110 64) for PCR strips or vortex centrifuge CVP-2 for PCR plates (Order No.: D 110 67). Dedicated protocols are available for this centrifuge.

Note: Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down the cap strip.

7. Spin the PCR tube strips for 30 seconds at 150 – 200 x g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 x g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1000 x g!

8. Place the samples in your PCR cycler and run the program as described above.

Note: For using any LightCycler 480 instrument, a special adapter (Order No.: Z 100 24) is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in column 1 and 12.



2.3 Data Interpretation

The amplification of the *stx1* gene is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The amplification of the *stx2* gene is analyzed in the fluorescence channel suitable for the detection of HEX labeled and the amplification of the *eae* gene is analyzed in the fluorescence channel suitable for the detection of ROX labeled probes. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for Cy5.

Compare the results from channel FAM (*stx1* gene), channel HEX (*stx2* gene), channel ROX (*eae* gene) and channel Cy5 (Internal Control) for each sample, and interpret the results as described in the table below.

Channel FAM	Channel HEX	Channel ROX	Channel Cy5	Result Interpretation
Positive	Positive	Positive	Positive or Negative	Positive for <i>stx1</i> , <i>stx2</i> and <i>eae</i>
Negative	Positive	Positive	Positive or Negative	Positive for <i>stx2</i> and <i>eae</i>
Positive	Negative	Positive	Positive or Negative	Positive for <i>stx1</i> and <i>eae</i>
Positive	Positive	Negative	Positive or Negative	Positive for <i>stx1</i> and <i>stx2</i>
Negative	Positive	Negative	Positive or Negative	Positive for <i>stx2</i>
Positive	Negative	Negative	Positive or Negative	Positive for <i>stx1</i>
Negative	Negative	Positive	Positive or Negative	Positive for <i>eae</i> - negative for STEC
Negative	Negative	Negative	Positive	Negative for STEC
Negative	Negative	Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of *stx1*-, *stx2*- and *eae* genes as well as Internal Control DNA in this multicolor experiment is a suitable calibration of the PCR instrument for channels FAM, HEX, ROX and Cy5. 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 or Cy5.
	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 Cy5 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[®] STEC Screening 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"> Store the lyophilized PCR mix always in the aluminum bag with the silica gel pad. Open Strip shortly before filling.

4. Additional Information on this Product

How this Product Works

The **foodproof**[®] STEC Screening 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 Cy5 channel, whereas the STEC DNA is detected in channels FAM (*stx1* gene), HEX (*stx2* gene) and ROX (*eae* gene). 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 STEC DNA in the sample. The **foodproof**[®] STEC Screening LyoKit minimizes contamination risk and contains all reagents (except for template

DNA) needed for the detection of STEC DNA. Primers and probes provide specific detection of STEC DNA in food samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

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 *stx1*-, *stx2*- and *eae* gene specific sequences.
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 sequence of the amplicon 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.

Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCR's. 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 STEC 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**® STEC Screening LyoKit, decontamination can be achieved with the provided reagents.

Background Information

Shiga toxin-producing *Escherichia coli* comprise strains of the bacterium *Escherichia coli* that, when infecting humans, have been linked with the severe complication hemolytic-uremic syndrome (HUS). They are known by a number of names, including enterohemorrhagic *E. coli* (EHEC), Shiga-like toxin-producing *E. coli* (STEC), hemolytic uremic syndrome-associated enterohemorrhagic *E. coli* (HUSEC) and verocytotoxin- or verotoxin-producing *E. coli* (VTEC).[1]

All these *E. coli* strains produce Shiga-like toxin (also known as verotoxin), a major cause of foodborne illness. These are distinguished from other pathotypes of intestinal pathogenic *E. coli* including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).

For both Shiga toxin-coding genes, *stx1* and *stx2*, several variants exist, namely *stx1*, *stx1c* and *stx1d* as well as *stx2*, *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g*. The **foodproof**® STEC Screening LyoKit detects all of these gene variants.

Detection of Shiga toxin-producing *Escherichia coli* with the **foodproof**® STEC Screening LyoKit is in accordance with ISO/TS 13136 [2] and USDA-FSIS method MLG 5C.00 [3]. These reference methods contain also a screen for the *eae* gene in addition to the *stx* genes.

References

1. Karch H, Tarr P, Bielaszewska M (2005). "Enterohaemorrhagic *Escherichia coli* in human medicine.". *Int J Med Microbiol* **295** (6-7): 405–18.
2. ISO/TS 13136:2012 "Microbiology of food and animal feed —Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens — Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups".
3. FSIS Microbiology Laboratory Guidebook (MLG) MLG 5C.00, "Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STECs) from Meat Products and Carcass and Environmental Sponges, 2019.



BIOTECON Diagnostics

Quality Control

The **foodproof**[®] STEC Screening LyoKit is function tested using the LightCycler[®] 480 System.

5. Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License

License Notice

The purchase price of this product includes limited, nontransferable rights under U.S. 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 trademark of BIOTECON Diagnostics GmbH.

Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions or experience any problems with our products, please contact our

HELPDESK



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Monday - Friday,
9:00 am - 5:00 pm CET

or



support@bc-diagnostics.com

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.

6. Change Index

Version 1, September 2013

First version of the package insert.

Version 2, March 2017

License Notice changed.

Introduction of vortex centrifuges into the PCR Setup Procedure.

Version 3, October 2020

Addition of AOAC certification information.

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