STEC qPCR Screening Kit

Kit Components

Component	Cat. No. E0480-01 96 reactions of 25 µl
STEC Master Mix	2 x 960 µl
PC – positive control	1 x 100 µl
Water, nuclease free	1 x 500 µl

Storage

Store the components at -20°C in the dark.
Repeated thawing and freezing (> 2x) should be avoided, as this may reduce the sensitivity.

Description

- STEC qPCR Screening Kit is a ready to use system for the detection of Shiga toxin-producing *Escherichia coli* (STEC) in food and environmental samples after enrichment.
- The kit contains reagents and primers for the amplification of specific regions of *E. coli* DNA. STECs are identified based on the presence of the Shiga toxin genes (*stx1* or *stx2*, including the *stx2f* subtype). The intimin gene (*eae*) is an additional virulence marker. The detection of the STEC amplicons is based on using the specific dual-labelled probes. The *stx1/stx2* genes are detected simultaneously in the FAM channel, while the *eae* gene in the Cy5 channel.
- In addition, the kit contains a further heterologous amplification system to monitor the proper DNA amplification and identify possible PCR inhibition simultaneously. The internal control system consists of primers and the HEX-labelled probe present in STEC Master Mix and exogenous DNA fragment. The amplification of the inner control does not interfere with the amplification of the specific STEC DNA and confirms PCR reaction correctness.
- STEC Master Mix contains onTaq DNA Polymerase, optimized reaction buffer, ROX and dNTPs.
- onTaq DNA Polymerase is a modified “hot start” enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated for 15 minutes.
- ROX passive reference dye included in the STEC Master Mix allows fluorescence normalization on certain cyclers. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Agilent. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument.
- DNA isolation should be performed with dedicated, commercially available kits using silica/magnetic beads technology.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Protocol

Preparation of PCR Reaction

Component	Negative Control	Sample DNA	Positive Control
STEC Master Mix	20 µl	20 µl	20 µl
PC — positive control	-	-	5 µl
Extracted DNA		5 µl	
Water, nuclease free	5 µl	-	-
Total volume	25 µl	25 µl	25 µl

Notes

1. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex and briefly centrifuge all solutions. Keep STEC Master Mix on ice.
3. Set up PCR reactions at room temperature according to the table above. Use of STEC Master Mix allows room temperature reaction setup.
4. Aliquot 20 µl of the reaction mixture into tubes or PCR plate wells and add:
   - 5 µl of water (negative control),
   - 5 µl of DNA (≤500 ng/reaction) purified according to the DNA isolation procedure (sample DNA),
   - 5 µl of PC (positive control).
5. We recommend to include at least one positive and one negative control in each PCR run. The negative control should be prepared first, followed by the DNA samples, and finally the positive control to minimize the risk of contamination.
6. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
7. Place the samples in the cycler and start the program.
8. Perform data analysis.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Thermal Cycling Conditions

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	15 min	1
Denaturation	95°C	15 s	40
Annealing/Extension	60°C	60 s	
Cooling	4°C	Indefinite	1

Notes

1. Run PCR reactions using standard ramp rate (standard mode).
2. During the initial denaturation step onTaq DNA Polymerase is activated. onTaq DNA Polymerase requires 15 min incubation at 95°C to restore activity.
3. Fluorescence detection should be set at the end of the extension step and executed using FAM, CY5 and HEX channels. FAM and CY5 channels detect STEC DNA and HEX channels detect the inner control.

Results Interpretation

Sample type	FAM channel	CY5 channel	HEX channel	Result
Negative Control	-	-	+	correct
Positive Control, PC	+	+	+	correct
DNA 1	-	-	+	negative
DNA 2	+	+	+/-	positive for stx1/stx2 and eae*
DNA 3	+	-	+/-	positive for stx1/stx2, negative for eae**
DNA 4	-	+	+/-	negative for stx1/stx2, positive for eae
DNA 5	-	-	-	incorrect

* test with STEC qPCR Identification Kit
** test with STEC qPCR Identification Kit, if required

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Notes

1. A test can be considered valid/correct if:
- negative control gives a signal only in the HEX channel,
- positive control gives a signal in the FAM, CY5 and HEX channels.
2. In some circumstances, when the reaction is strongly positive in FAM and/or Cy5 channel, the sample may give a weak or no signal in the HEX channel. This phenomenon is caused by competition between the amplification reactions of the sample DNA and the internal control DNA.
3. If a positive reaction occurs only in the FAM or Cy5 channel and the conditions in point 1 are met, the sample should be handled in accordance with applicable internal procedures.
4. The presence of a fluorescence signal in the HEX channel for a given sample indicates that the DNA amplification was correct and the PCR reaction was not inhibited. Very high CT values (> 34) in the HEX channel may indicate a partial inhibition of the PCR reaction. The complete absence of a signal in the HEX channel with the simultaneous lack of a signal in the FAM and CY5 channels may indicate complete inhibition of the PCR reaction. In this case, it is recommended to dilute the sample 10 times or repeat the DNA isolation and rerun the PCR reaction.
5. Lack of signal for each channel in the well indicates an error in PCR preparation.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23