Via Sant'Anna 131/135
61030 Cartoceto PU (IT)
Telephone + 39 0721830605
FAX +39 0721837154
e-mail: info@diatheva.com
www.diatheva.com

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

E. coli O104 FLUO kit MBK0085 (*E. coli* O104:H4)

25 reactions

INTENDED USE

The kit can be used for the identification of *Escherichia coli* O104 by Real-time PCR.

INTRODUCTION

E. coli is a bacterium that is found in the intestines of healthy humans and animals, and which is part of the normal bacterial flora. However, some *E. coli* strains can cause diseases and lead to serious infection. VTEC (verocytotoxin-producing Escherichia coli) is a group of pathogenic *E. coli* bacteria that can cause bloody diarrhoea and haemolytic uremic syndrome in humans, a serious condition that can lead to kidney failure and be fatal. A virulent and rare strain of VTEC, O104:H4, caused the 2011 E. coli outbreak in France and Germany. This is a rare EHEC strains or EAEC that has acquired the Shiga toxin gene. The outbreak in 2011 has causes major attention resulting in enhanced food security monitoring.

PRODUCT DESCRIPTION

The *E. coli* O104 FLUO kit is a qPCR assay developed to identify the pathogen by the amplification of a serotype related gene *wzx*.
The kit provide a ready to use PCR mix containing an Internal Control to assess the efficiency of amplification reaction revealing the presence of inhibitory factors in the sample. The kit also contains Positive PCR Control.

KIT CONTENTS

The kit is for 25 reactions

Component	Volume
E. coli O104 PCR mix	1 x 500µl
5U/µl Hot rescue DNA polymerase	1 x 7 µl
ROX solution	1 x 5 µl
Dilution Buffer	1 x 50 µl
Positive PCR Control (*E. coli* O104 genomic DNA)	1 x 50 µl
Negative PCR Control	1 x 100 µl

ADDITIONAL EQUIPMENT

- Micropipettes and filter tips
- Vortex
- Micro-centrifuge
- Real-time PCR thermal cycler
- Powder free gloves
- 1.5 ml tubes
- PCR tubes or PCR plates, sealing tapes and caps compatible with the PCR instrument

STORAGE

Store the kit at -20°C, protect from light. If properly stored, see the expiration date for the stability of the kit.

PRECAUTIONS

The test must be performed by specialised, trained and authorised staff.

- Do not use reagents after the expiry date printed on the label
- Thaw all components and samples at room temperature and then thawed, mix the components and centrifuges briefly
- It is suggested to analyse each samples and standards in duplicated

- Use calibrated pipettes and filter tips
- Use gloves (powder free) during the whole procedure
- Change gloves often, especially if you suspect a possible contamination of them
- It is suggested to provide separate and dedicated spaces, material and equipment for pre- and post-PCR amplification stages
- Clean working space periodically with at least 5% sodium hypochlorite or other decontaminant agent

PROCEDURE

1. SAMPLE PREPARATION

1.1. BACTERIAL COLONY

Real-time PCR could be used for colony confirmation.
• Dispense 100 µl of sterile water in a 1.5 ml tube and dissolve the colony using a sterile loop.
• Boil the sample for 10 minutes.
• Centrifuge at 14 000 rpm for 10 minutes.
• Transfer 50 µl of supernatant in a new 1.5 ml tube taking care to do not disrupt the pellet.
• Vortex, centrifuge briefly and use 1 µl of the DNA in the real-time PCR reaction.

2. PROGRAM SETUP

Program PCR instrument before preparing the reaction mix.
The kit has been optimized to be used with Rotor-Gene Q and ABI 7500 thermal cyclers. Otherwise it could be used also with other instruments, in this case please contact technical service of Diatheva.
• Program the real-time PCR instrument with the following thermal profile:

Step	Temperature and times	Cycles
Initial denaturation	95°C 10 min	1 X
Denaturation	95°C 15 sec	40 X*
Annealing-extension	60°C 60 sec	

* 45 cycles are required for instruments with peltier block (i.e. QS5, ABI 7500)
Fluorescence is detected during annealing-extension step on red channel (CY5 dye), yellow channel (VIC dye).

For Rotor-Gene Q instrument that allows the gain optimization on the acquiring channels, set the gain optimization on NTC sample (tube position 1).

• Select ROX as passive reference dye for instruments that require it (i.e. Applied Biosystems).

3. PCR MIX PREPARATION

All experiments should include a Negative PCR Control (NTC-No Template Control), containing all the components of the reaction except for the template. This enables detection of potential contamination. A Positive PCR control should be also included in each run.

• Thaw the components protect from light. Vortex 15" and centrifuge briefly.
• Upon first use of E. coli O104 PCR mix, <u>for instruments that require a passive reference dye</u>, it is necessary to add the ROX Solution:
-Low Rox: ABI 7500, Quantstudio5,
-High Rox: StepOne Plus
-No Rox: Rotor-Gene Q, CFX96 Biorad, MiniOpticon Biorad

• The E. coli O104 PCR mix should be completed immediately before the use according to the instructions below:
-<u>For Low Rox instruments</u> → add 45 µl of the Dilution Buffer to the vial containing the 5 µl ROX Solution and vortex for 30". Proceed by completing the E. coli O104 mix with the addition of 1.4 µL diluted ROX Solution.
-<u>For High Rox instruments</u> → the ROX Solution provided in the kit is ready to-use (no dilution is required). Proceed by completing the E. coli O104 PCR mix with the addition of 1.56 µL ROX solution.

• Vortex for 15" the prepared E. coli O104 PCR mix and centrifuge briefly.

- After the completion of the mix by ROX, in one sterile 1.5 ml tube prepare amplification reaction mix needed for each sample to be tested following the pipette scheme below:

	1 sample*
PCR mix	19.8 µl mix + 0.2 µl Hot rescue DNA Polymerase

* For the analysis of more than one sample, simply multiply the volumes of mix and Hot rescue DNA polymerase for the number of samples to be tested considering the Positive and Negative PCR control plus one additional reaction to cover pipetting losses.

- Vortex for 15" the prepared E. coli O104 PCR mix and centrifuge briefly.
- Aliquot 20 μl of E. coli O104 PCR mix in 0.2 ml tubes or in the plate prepared for the experiment.
- Add 5 μl of Negative PCR Control into NTC.
- In a separate area, add 1 μl of DNA samples to be tested and if necessary add DNAse free water to obtain a final reaction volume of 25 μl, into the corresponding PCR tubes or wells containing amplification mixes. If volumes greater than 1 μl are used, please consider to adjust the final volume to 25 μl.
- Add 5 μl of Positive PCR Control into the corresponding PCR tube or well containing amplification mixe.

Note: for PCR instrument with 96 wells block check that the reagents are at the bottom of each well, if not, briefly centrifuge at 800 x g for 1 minute.

5. DATA ANALYSIS

The analysis of the results should be done with the program of the PCR instrument, please refer to the manual for detailed information. Set the baseline and threshold values. Some software perform the data analysis automatically in this case it is advisable to check these settings. For a manual data analysis, analyze the PCR file for the two fluorophores separately. For a correct definition of the threshold it is necessary to select a value distinction from the background after the linear phase growth.

6. INTERPRETATION OF RESULTS

Controls
Before proceeding with the analysis of the results, check the validity of controls. If result differ from those indicated in the table below the PCR run is not valid.

	E. coli O104 red channel	Internal amplification control yellow channel
Negative PCR Control	No amplification	20<Ct<35**
Positive PCR Control	20<Ct<30**	Non significant*

**Ct values obtained using Quantstudio5 and ABI 7500 instrument.
*usually no amplification expected

Samples
Check that the curves are typical amplification curves. If the Ct value in the red channel is ≤10, verify in the raw data that the curve is a regular amplification curve. If correct the sample could be considered positive for *E. coli O104.*

E. coli O104 **result**	**Internal amplification control**	**Results and interpretation**
Ct≥10	Non significant	Sample positive for E. coli O104
No amplification	20<Ct≤35**	Sample negative for E. coli O104
	No amplification	Inhibition***
	Ct>35**	Inhibition***

**Ct values obtained using Quantstudio5 and ABI7500 instrument.
***Dilute DNA sample and repeat the PCR amplification.

REFERENCES

EFSA. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding p athogenicity assessment. EFSA Journal 2013;11(4):3138.