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Enteroaggregative *E. coli* kit MBK0084 (AggR – AaiC)

20 reactions

INTENDED USE

The kit could be used for the detection of genetic markers (aggR and aaiC) characteristic for Enteroaggregative E. coli (EAEC) by Real-time PCR.

INTRODUCTION

Enteroaggregative *Escherichia coli* has been cited as an important childhood enteric pathogen worldwide (Jensen et al., 2014). A recent large and lethal German outbreak caused by a Shiga toxin (Stx)-producing EAEC, brought attention to this pathogen that did not possess the eae gene, encoding the adherence factor intimin, but possessed genes that are typical of the EAEC group (Scheutz et al., 2011). This matter has highlighted the importance to screen foodstuffs for EAEC too by the detection of targets designed on the aggR and aaiC genes, which represent genetic markers characteristic for this group of pathogenic E. coli (EFSA, 2013).

PRODUCT DESCRIPTION

The kit is qPCR assay to detect Enteroaggregative *E. coli* by the amplification of a specific sequences of two genetic markers of typical EAEC: the plasmid located gene aggR, coding for a transcription regulator, and the chromosomal gene aaiC, which is part of the aai gene cluster, encoding a type VI secretion system, with the use of fluorescent-labelled probe.
The kits 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 Enteroaggregative E. coli kit also contains Positive PCR Control.

KIT CONTENTS

The kit is for 20 reactions

Component	Volume
Enteroaggregative E. coli PCR mix	1 x 400µl
Hot rescue DNA polymerase 5U/µl	1 x 5 µl
ROX solution	1 x 5 µl
Dilution Buffer	1 x 100 µl
Positive PCR Control (aggR and aaiC)	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 analyze 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.
NOTE: the final reaction volume is 25 µl. Please add DNAse free water to adjust the final volume to 25µl.

For different application please contact Diatheva technical service.

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	40X*
Annealing-extension	60°C 60 sec	

* 45 cycles are required for instruments with peltier block (i.e. ABI 7500)
Fluorescence is detected during annealing-extension step on green channel (FAM dye), yellow channel (VIC dye) and red channel (Cy5 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 (es. Applied Biosystems).

3. PCR MIX PREPARATION

All detection 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 Enteroaggregative PCR mix, <u>for instruments that require a passive reference dye</u>, it is necessary to add the ROX Solution:
-Low Rox: ABI 7500, 7500 Fast,
-High Rox: StepOne, StepOne Plus
-No Rox: Rotor-Gene Q, CFX96 Biorad, MiniOpticon Biorad

• The E. coli Enteroaggregative 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 enteroaggregative mix with the addition of 1.10 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 enteroaggregative mix with the addition of 1.25 µL ROX solution.

- After the completion of the mix by adding the ROX solution, 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 reactions to cover pipetting losses.

- Vortex for 15" the prepared E. coli Enteroaggregative PCR mix and centrifuge briefly.
- Aliquot 20 µl of E. coli Enteroaggregative 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 greater volumes of samples are added to the PCR mix please consider to adjust the final volume to 25 µl.
- Add 5 µl of Positive PCR Control into the corresponding PCR tubes or wells containing amplification mixes.

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 differs from those indicated in the table below the PCR run is not valid.

	aggR green channel	*aaiC* red channel	Internal amplification control yellow channel
Negative PCR Control	No amplification	No amplification	20<Ct<35**
Positive PCR Control*	22<Ct<32**	22<Ct<32**	Non significant*

**Ct values obtained using ABI 7500 and QuantStudio5 instrument.
*usually no amplification expected

Samples
Check that the curves are typical amplification curves. If the Ct value in the green channel is ≤10, verify in the raw data that the curve is a regular amplification curve.

aggR result	*aaiC* result	Internal amplification control	Results and interpretation
Ct≥10	-	Non significant	Sample positive for *aggR*
-	Ct≥10	Non significant	Sample positive for *aaiC*
Ct≥10	Ct≥10	Non significant	Sample positive for *aggR* and *aaiC*
No amplification	No amplification	20<Ct≤35**	Sample negative for *aggR* and *aaiC*
		No amplification	Inhibition***
		Ct>35**	Inhibition***

**Ct values obtained using ABI 7500 and QuantStudio5 instrument.
***Dilute DNA sample and repeat the PCR amplification.

REFERENCES

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