DEC PCR Kit

DEC PCR Kit for PCR detection of diarrhoeagenic *E. coli* (DEC)

For *in vitro* diagnostic use only

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Application

The DEC PCR Kit is to be used for *in vitro* diagnostic PCR detection of diarrhoeagenic *E. coli* (DEC)[1].

Description

The DEC PCR Kit contains primer mix, two PCR positive DNA controls, PCR ReadyMix (including loading buffer), TE-buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8) and 10% Chelex-100 in TE-buffer.

The primer mix contains 8 primer pairs directed towards the following genes: intimin (*eae*), verocytotoxin 1 (*vtx1*), verocytotoxin 2 (*vtx2*), heat stable enterotoxin, human variant (*estA-human*), heat stable enterotoxin, porcine variant (*estA-porcine*), heat labile enterotoxin (*eltA*), invasive plasmid antigen (*ipaH*) and *16S* rDNA (positive internal control). All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'- hydroxyl ends. The individual concentration of each primer pair is adjusted for optimal performance in the multiplex PCR. The primer mix contains 700 µL enough to perform 100 PCR tests.

The PCR positive control set includes two different DNA templates. Control 1 consists of purified DNA from a non-pathogenic *E. coli* plus DNA from a strain with the *eae*, *vtx1* and *vtx2* genes. Control 2 contains a mixture of purified DNA from a non-pathogenic *E. coli* strain, a strain with the *ipaH* gene and a strain with the *eltA* and *estA* genes. Each control DNA vial contains 150 µL corresponding to at least 25 PCR tests.

Background

The most important DECs are: verocytotoxin producing *E. coli* (VTEC), enteropathogenic *E. coli* (EPEC), attaching and effacing *E. coli* (A/EEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC)[2]. The genes used for identification of the particular DEC and the amplicon sizes are listed below.

Gene	DEC	Amplicon size (bp)
16S rDNA[a]	-	1062
ipaH[b]	EIEC	647
eltA	ETEC	479
vtx2	VTEC	420
eae	EPEC, A/EEC[c]	377
vtx1[d]	VTEC	260
estA-porcine[e]	ETEC	160
estA-human[e]	ETEC	151

a) Amplifies a fragment from most Gram-negative bacteria, allowing an evaluation of the PCR.
b) Might also be present in *Shigella* spp.
c) EPEC and A/EEC distinction is based on serotype.
d) Might also be present in *Shigella dysenteriae I.*
e) Amplicon sizes cannot be differentiated under standard agarose gelelectrophoresis.

Materials required but not provided

Agar plate
1.5 – 2.0% agarose gel
100 bp DNA marker

Procedure

1. If running only a few PCRs at a time, dispense the primer mix and the PCR ReadyMix into several aliquots and use only one at a time.
2. Pick up to 10 plate-grown colonies and suspend them in a tube containing 200 µL 10% Chelex-100 .
3. Boil the suspension for 5 min. and centrifuge briefly (5 min. at app. 2200 x *g*).
4. Dilute 15.0 µL of supernatant in 100 µL TE-buffer and use 4.0 µL directly in the PCR.
5. Sample preparation and PCR setup should be performed in dedicated areas free of possible contamination. Prepare the total mastermix for the number of samples to be run and dispense 16.0 µL of the mixture in each tube. Each tube has to contain the following amount of reagents:

Mastermix:	
Polymerase mix	10.0 µL
Primer mix	6.0 µL
Total	16.0 µL

6. Add 4.0 µL of template DNA (sample or positive control) to each tube and mix. Prepare a negative control by adding 4.0 µL TE-buffer to one of the tubes.
7. Run the PCR amplification in a thermocycler at the following conditions.

Step	Temp. (ºC)	Time
Initial denaturation	95	2 min
35 cycles of:		
Denaturation	94	50 sec
Annealing	62	40 sec
Extension	72	50 sec
Final extension	72	3 min

8. Run 10-15 µL of each completed PCR reaction in separate wells on an agarose gel (1.5-2.0%) capable of separating the particular amplicon sizes.

Interpretation of the analysis results

The figure below shows the PCR results containing a variety of different templates compared to a 100 bp DNA marker.

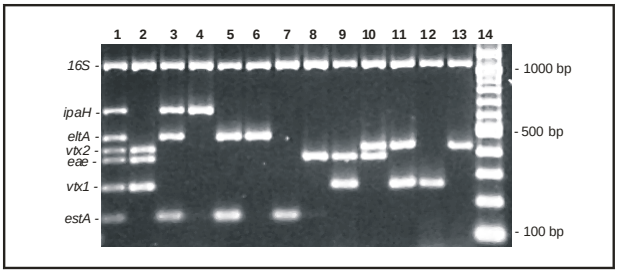

Figure. Analysis of 13 different templates containing: lane 1: est*A*, *vtx1*, *eae*, *vtx2*, *eltA* and *ipaH*, lane 2: *vtx1*, *vtx2* and *eae*, lane 3: est*A*, *eltA* and *ipaH*, lane 4: *ipaH*, lane 5: est*A* and *eltA*, lane 6: *eltA*, lane 7: est*A*, lane 8: *eae*, lane 9: *vtx1*and *eae*, lane 10: *vtx2* and *eae*, lane 11: *vtx1* and *vtx2*, lane 12: *vtx1*, lane 13: *vtx2* and lane 14: 100 bp DNA marker.

Specificity

A total of 142 reference strains from "The International *Escherichia* and *Klebsiella* Centre (WHO)", Statens Serum Institut, Denmark were tested by the multiplex PCR method. The strains represent a broad variety of different serotypes and virulence gene combinations. Compared to a DNA hybridisation technique targeting the same genes, the PCR method was 100% specific. Also tested were 13 non-*E. coli* species, all of which only produced the *16S* rDNA control band.

Storage and shelf life

The PCR ReadyMix, the DEC primer mix and the DNA controls should be stored at ÷20 ºC. Dispense the reagents into several aliquots and store them at ÷20°C. The DEC primer mix and the controls in use can be stored at 4-8 °C for up to 2 weeks. Store 10% Chelex-100 and TE-buffer at room temperature.
The expiry date of the kit is printed on the label.

References

1. Persson, S., Olsen, K. E. P., Scheutz, F., Krogfelt, K. A. and Gerner-Smidt, P. 2007. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clin Microbiol Infect. 13:516-524.
2. Nataro, J. P. and J. B. Kaper. 1998. Diarrhoeagenic Escherichia coli. Clin. Microbiol Rev. 11:142-201.

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