

BactoReal® Kit *E. coli* Typing



For veterinary use only

Series BactoReal® Kit *E. coli* Typing

Kit name	Detection assay(s) for	Pathogen	IPC	Order no.	Reactions
BactoReal® Kit <i>E. coli</i> Typing stx1 & stx2 (STEC)	stx1 & stx2	FAM + VIC/HEX channel	Cy5 channel	DVEB04013	100
				DVEB04053	50
BactoReal® Kit <i>E. coli</i> Typing eae & ehxA (EHEC, EPEC)	eae & ehxA	FAM + VIC/HEX channel	Cy5 channel	DVEB04113	100
				DVEB04153	50
BactoReal® Kit <i>E. coli</i> Typing eae (EHEC or EPEC)	eae	FAM channel	Cy5 channel	DVEB04313	100
				DVEB04353	50
BactoReal® Kit <i>E. coli</i> Typing ehxA (EHEC)	ehxA	VIC/HEX channel	Cy5 channel	DVEB04413	100
				DVEB04453	50

Kits contents:

- Detection assays for stx, eae or ehxA (see above)
- Detection assay for internal positive control (IPC, control of amplification)
- DNA reaction mix (contains uracil-N glycosylase, UNG)
- Positive controls
- Water

Background: *Escherichia coli* is a gram negative bacterium that is a member of the normal gut flora of humans and animals. Most *E. coli* strains are harmless, but some serotypes carry virulence factors which enable them to be pathogenic in target hosts. These pathogenic *E. coli* are implicated in a number of diseases including diarrhoea, septicaemia and urinary tract infections in humans and animals.

Enteropathogenic *Escherichia coli* (EPEC) produces an outer membrane protein called intimin, encoded by *eae* and mediating intestinal cell attachment. Intimin is a virulence factor (adhesin) of EPEC and EHEC strains. Furthermore, EPEC are classified into typical and atypical strains based on the presence of the plasmid *E. coli* adherence factor (EAF). Typical EPEC strains are rarely isolated from animals, and humans are the major natural reservoir. The reservoir for atypical EPEC can be both animals and humans. By definition all EPEC lack genes to produce shiga toxin (stx).

Shiga toxin (verotoxin) producing *Escherichia coli* (STEC) is recognized as an important human diarrheal pathogen. Swine and cattle play an important role as a carrier of STEC. The ability of STEC to cause illness is due to the production of Shiga toxins Stx1 and/or Stx2 (encoded by *stx1* and *stx2* genes).

Enterohaemorrhagic *Escherichia coli* (EHEC) can cause severe foodborne disease. EHEC is also positive for *stx1* and/or *stx2* but also produces either intimin (*eae*) or hemolysin (*ehxA*).

Description: BactoReal® Kit *E. coli* Typing is a series of different real-time PCR kits for detection of virulence-associated genes of *E. coli*. Depending on which kit and which combinations of kits are used, they allow the detection of DNA of enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC) and of Shiga toxin (verotoxin) producing *E. coli* (STEC) purified from stool or other sample material (e.g. with the QIAamp DNA Stool Mini Kit). Furthermore, isolated bacterial colonies can be picket from the plate, isolated e.g. with InstaGene™ Matrix (Bio-Rad) and analysed.

Stx1 is detected in FAM channel. Stx2 is detected in VIC/HEX channel. Eae is detected in FAM channel. EhxA is detected in VIC/HEX channel. Assays for stx1 and stx2, as well as assays for eae and ehxA can be combined in a multiplex PCR.

Typing of pathogenic *Escherichia coli* according to virulence-associated genes:

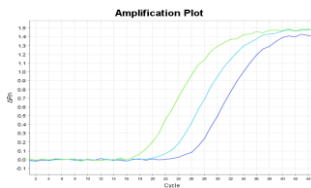
Genes and detection channel	EPEC	EHEC	STEC
eae (FAM)	positive	eae and/or ehxA positive	negative
ehxA (VIC/HEX)	negative		negative
stx1 (FAM)	negative	stx1 and/or stx2 positive	stx1 and/or stx2 positive
stx2 (VIC/HEX)	negative		

eae: intimin **ehxA:** enterohemolysin **stx:** Shiga toxin (also known as Shiga-like toxin, SLT or verotoxin, vtx)

PCR-platforms: Kits are developed and validated for the ABI PRISM® 7500 instrument (Life Technologies), LightCycler® 480 (Roche) and Mx3005P® QPCR System (Agilent), but are also suitable for other real-time PCR instruments.

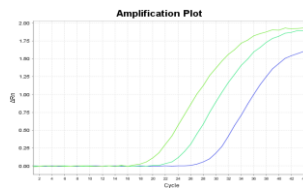
Sensitivity and specificity: The analytical sensitivity is 15 target copies for stx1 and stx2 per PCR reaction and approx. 10 target copies for eae or ehxA per PCR reaction. The stx2 variant stx2f is not detected.

Detection of **stx1**



ABI Prism® 7500: FAM channel
1:10 serial dilution of *E. coli* stx1

Detection of **stx2**



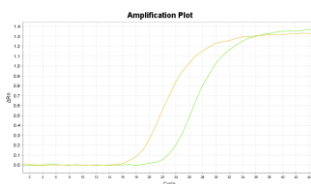
ABI Prism® 7500: VIC channel
1:10 serial dilution of *E. coli* stx2

Detection of **internal positive control**



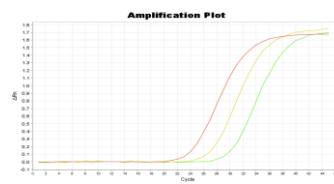
ABI Prism® 7500: Cy5 channel
Internal positive control CR-3

Detection of **eae**



ABI Prism® 7500: FAM channel
1:10 dilution of *E. coli* eae

Detection of **ehxA**



ABI Prism® 7500: VIC channel
1:10 dilution of *E. coli* ehxA

BactoReal®, MycoReal, ParoReal and ViroReal® Kits run with the same thermal cycling conditions. RNA and DNA material can be analysed in one PCR run.

For further information on our products please visit our homepage (www.ingenetix.com)