BactoReal® Kit *E. coli & Klebsiella*

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *E. coli & Klebsiella*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUS00153	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of *E. coli, Klebsiella* and of internal DNA positive control (IPC)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction mix (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- DNA positive control for *E. coli, Klebsiella pneumoniae*
- Nuclease-free water

Pathogen information: *Escherichia coli* is a Gram-negative bacterium inhabiting the normal intestinal flora. Most *E. coli* strains are non-pathogenic but some strains of this species can cause disease such as urinary tract infections, gastrointestinal infections, or sepsis.
Klebsiella species are Gram-negative, facultatively anaerobic bacteria found ubiquitously in the environment. They are part of the healthy gut flora in humans. Outside the intestine they can lead to infections such as pneumonia and sepsis. Some strains show antibiotic resistance and are therefore difficult to treat. Both *E. coli* and *Klebsiella* are microorganisms responsible for nosocomial infections.

Intended purpose: BactoReal® Kit *E. coli & Klebsiella* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA of *Escherichia coli* (dxs gene), *Klebsiella pneumoniae, K. quasipneumoniae, K. variicola, K. oxytoca* and *K. aerogenes* (khe gene).
Proper specimens are DNA extracts isolated from samples of human EDTA blood, aspirates, CSF and biopsies.
In combination with other diagnostic tests such as culture the test supports a rapid and specific diagnosis for patients suspected of having a bacterial infection. Results must be interpreted in context of the overall picture and other clinical parameters. The diagnosis of sepsis must not be based solely on BactoReal® Kit *E. coli & Klebsiella*.
The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.
E. coli DNA is detected in FAM channel and *Klebsiella* in VIC channel. The internal DNA positive control (DNA IPC) is detected in Cy5 channel and is used as DNA extraction as well as PCR inhibition control. The DNA IPC target (artificial DNA) is extracted with the sample.

PCR-platforms: This test has been validated with the ABI® 7500 Fast Real-time PCR System (fast cycle parameters are not supported, Thermo Fisher Scientific) and with the cobas z 480 Analyzer (Roche). It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, VIC and Cy5 channel (e.g., QuantStudio™ 5, QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), Mic instrument (bio molecular systems), LightCycler® 480 II (Roche)).

Performance data: The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 50 copies/reaction for *E. coli* and 9 copies/reaction for *Klebsiella*. A cut-off with Cq=35 must be defined for *E. coli*. This kit is specific for *E. coli* and *Klebsiella pneumoniae, K. quasipneumoniae, K. variicola, K. oxytoca* and *K. aerogenes.*
For clinical validation, 25 blood samples and 9 other specimens such as CSF, aspirates, and biopsies of patients with suspected bacterial infection were analyzed (see Table 1).

Table 1 Statistical clinical evaluation for blood samples and other specimens

	Sensitivity		Specificity		NPV		PPV		Prevalence		Accuracy	
	Blood	Other	Blood	Other	Blood	Other	Blood	Other	Blood	Other	Blood	Other
E. coli	100.0%	100.0%	81.3%	100.0%	100.0%	100.0%	75.0%	100.0%	36.0%	22.2%	88.0%	100.0%
Klebsiella	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	44.0%	22.2%	100.0%	100.0%

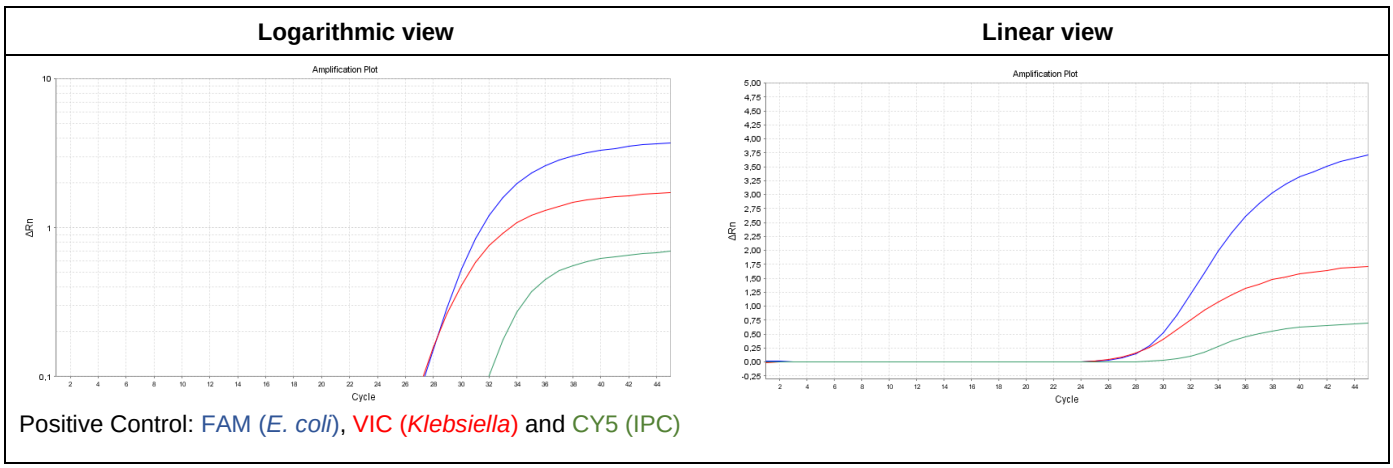

Figure 1 Performance of BactoReal® Kit *E. coli & Klebsiella*

Further tests for diagnosis of sepsis, which can be combined with BactoReal® Kit *E. coli & Klebsiella*:
BactoReal® Kit *E. coli* & *Klebsiella*, order no.: DHUS00153
BactoReal® Kit *Staphylococcus* spp. & *S. aureus*, order no.: DHUS00253
BactoReal® Kit *S. pneumoniae* & *Streptococcus* spp., order no.: DHUS00353
BactoReal® Kit vanA & *Enterococcus* spp., order no.: DHUS00453
BactoReal® Kit *P. aeruginosa* & *Enterobacter* spp., order no.: DHUS00553
BactoReal® Kit *A. baumannii* & *P. mirabilis*, order no.: DHUS00653
MycoReal® Kit *Candida* & *A. fumigatus*, order no.: DHUF00153

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