BactoReal® Kit *Bordetella* Multiplex

Kit version 1.1

For *in vitro* diagnostic use only

BactoReal® Kit *Bordetella* Multiplex			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUB00453	50	FAM + VIC channel	Cy5 channel

Kit contents:

- Assay for detection of *Bordetella* 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 *B. pertussis* + *B. parapertussis*
- Nuclease-free water

Pathogen information: *Bordetella pertussis* is a gram-negative bacterium of the genus *Bordetella* and the causative agent of pertussis, an acute respiratory disease. Transmission occurs by droplet infection. *Bordetella parapertussis*, *Bordetella holmesii* and *Bordetella bronchiseptica* can cause a whooping cough-like illness with a milder clinical course. While *Bordetella pertussis* and *Bordetella parapertussis* only infect humans, *Bordetella bronchiseptica occurs* mainly in animals and is occasionally isolated from humans. The IS481 is a multicopy gene found up to 249 times in the genome of *B. pertussis.* The IS1001 is also multicopy gene, found up to 22 times in the genome of *B. parapertussis*.

Intended purpose: BactoReal® Kit *Bordetella* Multiplex is a non-automated CE-certified IVD real-time PCR test for the qualitative detection and identification of DNA of *Bordetella pertussis, Bordetella parapertussis, Bordetella holmesii* and some strains of *Bordetella bronchiseptica*. This test detects the insertion sequence IS481 of *B. pertussis, B. holmesii* and *B. bronchiseptica* as well as the insertion sequence IS1001 of *B. parapertussis*, *B. bronchiseptica* and *B. holmesii*. IS481 or IS1001 may be present in *B. bronchiseptica* in rare cases.
Proper specimens are DNA extracts isolated from samples of the human respiratory tract (sputum, BAL, nasal swabs, throat swabs, bronchial secretion, tracheal secretion).
This test is suitable for patients of all ages with suspected respiratory infection with *Bordetella* (causative agent of whooping cough and whooping cough-like infections) and is intended as an aid in the diagnosis of infection with this pathogen in combination with patient history and additional clinical information.
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.
In the fluorescence channel for FAM the insertion sequence IS481 of *B. pertussis, B. holmesii* and *B. bronchiseptica* (multicopy gene, found up to 249 times in the genome of *B. pertussis*) is detected. In the fluorescence channel for VIC the insertion sequence IS1001 of *B. parapertussis, B. holmesii* and *B. bronchiseptica* (multicopy gene, found up to 22 times in the genome of *B. parapertussis*) is detected. A probe-specific amplification-curve in the FAM channel and VIC channel indicates the amplification of *Bordetella holmesii* specific DNA.

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The internal DNA positive control (IPC) is detected in the fluorescence channel Cy5 and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for the DNA IPC (artificial target DNA) is added during sample extraction.

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 was also tested with a LightCycler® 480 II (Roche Diagnostics), QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific) and Mic instrument (bio molecular systems).
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 real-time PCR system (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), cobas z 480 Analyzer (Roche)).

Performance data: The LoD95% (smallest number of target DNA copies which can be detected in 95% of cases) is 0.06 genome copies/reaction for *B. pertussis* and 0.18 genome copies/reaction for *B. parapertussis*. This kit is specific for *B. pertussis, B. parapertussis, B. holmesii* and *B. bronchiseptica*, but cross reacts with *Achromobacter denitrificans.* Clinical validation was performed with 181 clinical samples (Table 1).

Table 1 Results of clinical validation

	Value	95% CI
Sensitivity	100.00%	95.55% to 100.00%
Specificity	100.00%	96.38% to 100.00%
NPV	100.00%	
PPV	100.00%	
Prevalence	44.75%	37.37% to 52.31%

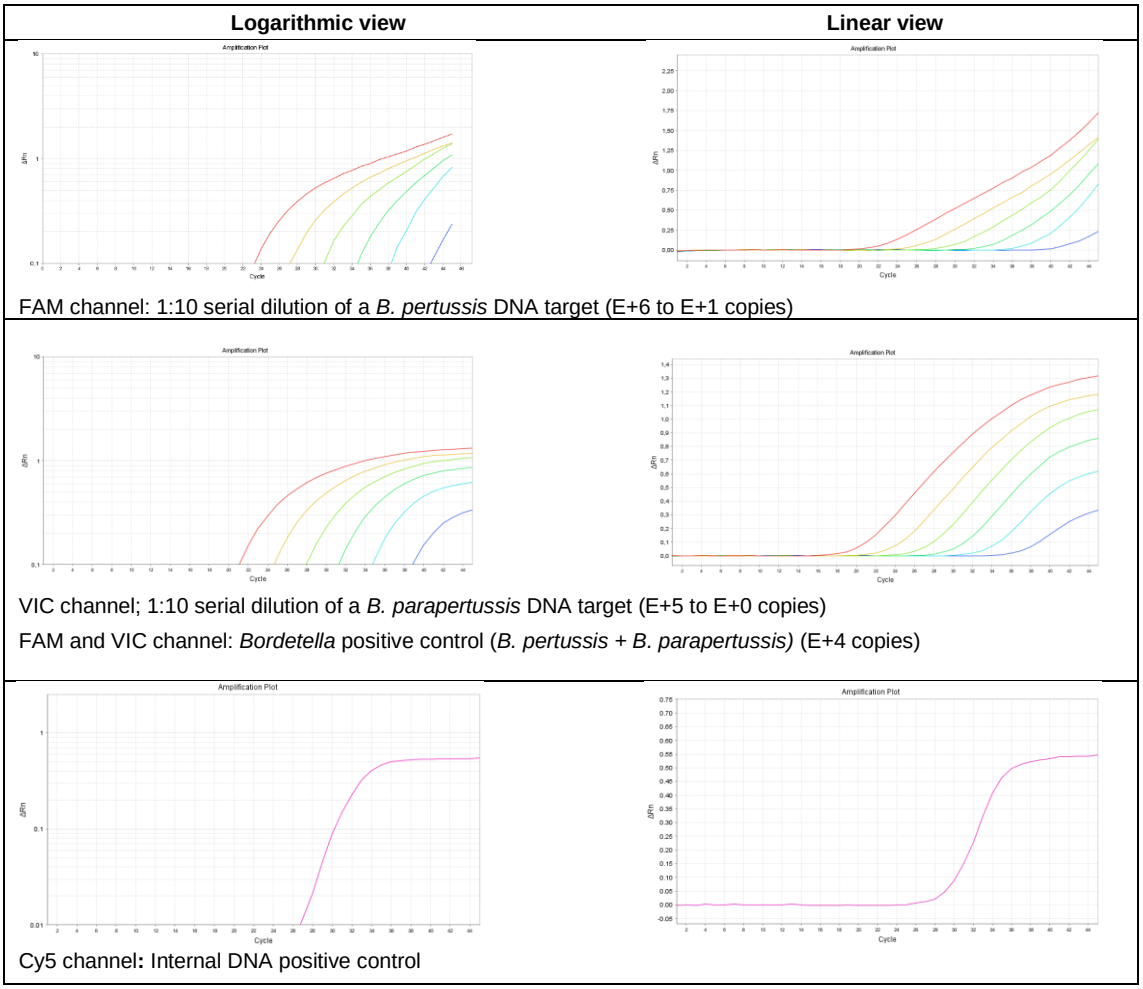

Figure 1 Performance of BactoReal® Kit *Bordetella* Multiplex