

# BactoReal<sup>®</sup> Kit

## *Bordetella bronchiseptica*

### Manual



For Research Use Only

**REF** DHUB02453

**Σ** 50 reactions



**ingenetix GmbH**  
Arsenalstraße 11  
1030 Vienna, Austria  
T +43(0)1 36 198 0 198  
F +43(0)1 36 198 0 199  
office@ingenetix.com  
www.ingenetix.com

## Index

2. Product description.....	3
3. Pathogen information.....	3
4. Contents of the kit, stability and storage .....	4
5. Additionally required materials and devices .....	4
6. Precautions and safety information.....	4
7. Limitations .....	5
8. Preparation of samples.....	5
9. Preparation of real-time PCR.....	5
9.1. Pipetting scheme .....	5
9.2. Programming of temperature profile.....	6
10. Interpretation of PCR-data .....	6
11. Troubleshooting.....	7
11.1. No signal in FAM channel and Cy5 channel with controls and sample .....	7
11.2. Valid results for controls, but no signal in FAM channel and Cy5 channel with sample .....	7
11.3. Signal in FAM channel in negative control.....	7
11.4. Signal in FAM channel in extraction negative control .....	7
12. Specifications and performance evaluation.....	8
12.1. Kit performance.....	8
12.2. Analytical sensitivity and linearity .....	9
12.3. Analytical specificity .....	9
13. References .....	9

## Explanation of symbols



Batch code



Use by



Catalogue number



Manufactured by



Contains sufficient for <n> tests



Store at



Corrosion, GHS05



Exclamation mark, GHS07

## 1. Intended use

BactoReal<sup>®</sup> Kit *Bordetella bronchiseptica* is a real-time PCR assay for detection of *B. bronchiseptica* DNA.

## 2. Product description

BactoReal<sup>®</sup> Kit *Bordetella bronchiseptica* detects the *bfrA* gene of *B. bronchiseptica*. This test allows the rapid and sensitive detection of DNA of *Bordetella bronchiseptica* from samples purified from the respiratory tract (e.g. with the QIAamp DNA Mini Kit).

A probe-specific amplification-curve in the FAM channel indicates the amplification of *B. bronchiseptica* specific DNA. An internal DNA positive control (DNA IPC) is detected in Cy5 channel and is used as DNA extraction as well as real-time PCR inhibition control. The target for the DNA IPC is extracted with the sample. This test has been validated with the Applied Biosystems<sup>®</sup> 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler<sup>®</sup> 480 Instrument II (Roche) and Mx3005P<sup>®</sup> QPCR System (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel.

The test is based on real-time PCR. A specific DNA sequence of the pathogen genome is detected and amplified. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplicates.

BactoReal<sup>®</sup>, ParoReal and ViroReal<sup>®</sup> Kits are optimized to run under the same thermal cycling conditions. RNA and DNA can be analysed in one run.

## 3. Pathogen information

*Bordetella bronchiseptica* is a gram-negative bacterium found as a normal inhabitant of the respiratory tract in a variety of animals such as dogs, pigs, some rodents, as well as humans. While it likely causes pulmonary infections in animals, it rarely provokes human disease. However, in immunocompromised humans (HIV-infection, organ transplantation), it may cause severe cases of pneumonia which require antibiotic treatment.

#### 4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
<i>Bordetella bronchiseptica</i> Assay Mix (green cap)	Primer and probe (FAM) for <i>B. bronchiseptica</i> detection	1 x 50 µl	-15 °C to -25 °C
DNA IPC-3 Assay Mix (yellow cap)	Primer and probe (Cy5) for DNA IPC detection	1 x 50 µl	-15 °C to -25 °C
DNA IPC Target (orange cap)	Target for DNA IPC (internal DNA positive control system)	1 x 100 µl	-15 °C to -25 °C
<i>Bordetella bronchiseptica</i> Positive Control (red cap)	DNA positive control (approx. 10,000 target copies/µl)	1 x 25 µl	-15 °C to -25 °C
DNA Reaction Mix (white cap)	2 x DNA reaction mix	1 x 500 µl	<b>-15 °C to -25 °C until first use, then at +4 °C</b>
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-15 °C to -25 °C

The components of BactoReal® Kit *Bordetella bronchiseptica* are stable until the expiry date stated on the label.

#### 5. Additionally required materials and devices

- Reagents and devices for DNA-extraction
- Nuclease-free water for dilution of DNA IPC Target
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

#### 6. Precautions and safety information

- Clean benches and devices periodically.
- Use sterile filter pipette tips.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
- Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
- Quality of DNA has a profound impact on the test performance. Ensure that the used DNA extraction system is compatible with real-time PCR technology.
- Always include a negative control per PCR-run (nuclease-free water instead of sample).
- For a valid interpretation of results, a negative control should be included during DNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with DNA of pathogen during extraction.
- Please note the expiry date of the kit.
- Do not interchange or mix reagents from kits with different lot numbers.
- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light. **Caution:** DNA IPC Target is stored in RNA/DNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, [www.ingenetix.com](http://www.ingenetix.com)).
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

## 7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate DNA extraction procedure.
- Test results may be affected by improper specimen collection, technical error, specimen mix-up or pathogen quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this kit highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.

## 8. Preparation of samples

Extract samples with a DNA extraction system compatible with real-time PCR technology. An extraction negative control should be included during DNA-extraction (e.g. extraction of water instead of sample material).

The **DNA IPC Target** has to be added during extraction. The DNA IPC is used as a control of DNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.

**Caution:** The DNA IPC Target must not be added directly to the sample material but has to be pipetted to the lysis buffer.

→ For an elution volume of 50-100 µl: Per sample, spike 1 µl DNA IPC Target into lysis buffer.

→ For an elution volume of >100 µl or when using an automated extraction system: Per sample, spike 2 µl DNA IPC Target into lysis buffer.

## 9. Preparation of real-time PCR

- Include one negative control (water), one positive control and one extraction negative control per PCR run.
- It is recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Thaw DNA samples on ice.
- Thaw kit components at room temperature. When thawed, mix gently the components, centrifuge briefly and keep on ice.
- Mix the DNA Reaction Mix to ensure homogeneity of solution.
- **Positive Control**
  - Use 1 µl of *Bordetella bronchiseptica* Positive Control + 4 µl nuclease-free water.
  - Pipette positive control at last.

### 9.1. Pipetting scheme

		<b>Per sample</b>
<b>Preparation of Master Mix</b> (mix well)	Nuclease-free Water*	3.0 µl
	DNA Reaction Mix	10.0 µl
	<i>Bordetella bronchiseptica</i> Assay Mix	1.0 µl
	DNA IPC-3 Assay Mix	1.0 µl
	<b>Total volume Master Mix</b>	<b>15.0 µl</b>
<b>Preparation of PCR</b>	Master Mix	15.0 µl
	DNA-Sample*	5.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

\*1-8 µl of the sample can be used. When using ≠ 5 µl sample, the volume of water has to be adjusted accordingly.

→ **If DNA IPC Target was not added during extraction:** Freshly dilute the DNA IPC Target 1:100 with nuclease-free water and add 1 µl per sample directly to the master mix.

**Caution:** The use of more than 1 µl diluted (1:100) DNA IPC Target per reaction causes inhibition of the real-time PCR reaction.

## 9.2. Programming of temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

**Select detection channel:** FAM-TAMRA, 530 nm (for *B. bronchiseptica*)  
Cy5-NONE, 667 nm (for DNA IPC-3)

**Select reference dye (passive reference):** ROX

**Sample Volume:** 20 µl

**Temperature Profile:**

Program 1	Program 2	Program 3
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°
50°C *2 min	95°C 20 sec	95°C 5 sec 60°C 1 min

For ABI PRISM® 7500:  
Ramp speed: "Standard"

For LightCycler® 480 instrument:  
Detection format: 2 Color Hydrolysis Probe

**\*Note:** If viral RNA should be also detected in the same PCR run, program 1 has to be prolonged to 15 min at 50°C. This temperature profile can be used for all BactoReal®, MycoReal, PoroReal and ViroReal® kits for the detection of DNA or RNA.

## 10. Interpretation of PCR-data

For analysis of PCR results select fluorescence display options 530 nm (FAM channel) for the *B. bronchiseptica* target and 667 nm (Cy5 channel) for the DNA IPC target. Samples with positive Ct or Cp-values are considered positive. Please additionally check the amplification-curves manually. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

	FAM channel <i>B. bronchiseptica</i> target	Cy5 channel DNA IPC target	Interpretation
Negative control	Negative	Negative / Positive <sup>1</sup>	Valid
Positive control	Positive	Negative / Positive <sup>1</sup>	Valid
Extraction negative control	Negative	Positive	Valid
Sample	Positive	Positive / Negative <sup>2</sup>	Positive
Sample	Negative	Positive	Negative <sup>3</sup>
Sample	Negative	Negative	Invalid

<sup>1</sup>Only positive if the DNA IPC Target was added at a 1:100 dilution directly to the master mix

<sup>2</sup>High pathogen load in the sample can lead to reduced or absent signal of DNA IPC

<sup>3</sup>The positive signal of the DNA IPC excludes a possible PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted DNA sample (see 11. Troubleshooting).

## 11. Troubleshooting

### 11.1. No signal in FAM channel and Cy5 channel with controls and sample

- Incorrect programming of the temperature profile or detection channels of the real-time PCR instrument.  
→ Compare temperature profile and programming of detection channels with the protocol.
- Incorrect configuration of PCR reaction.  
→ Check your work steps (see pipetting scheme) and repeat PCR, if necessary.

### 11.2. Valid results for controls, but no signal in FAM channel and Cy5 channel with sample

- Incorrect programming of detection channels with the sample.  
→ Compare programming of detection channels with protocol.
- If the DNA IPC Target was added during extraction:
  - PCR reaction has been inhibited.
  - DNA extraction has failed.
  - The undiluted DNA IPC Target has not been added to lysis buffer of sample.
  - The extracted sample has not been added to PCR reaction.  
→ No interpretation can be made. Make sure you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions. Check your work steps.

### 11.3. Signal in FAM channel in negative control

- A contamination occurred during preparation of PCR.  
→ Repeat PCR with new reagents in replicates.  
→ Strictly pipette positive control at last.  
→ Make sure that work space and instruments are cleaned at regular intervals.

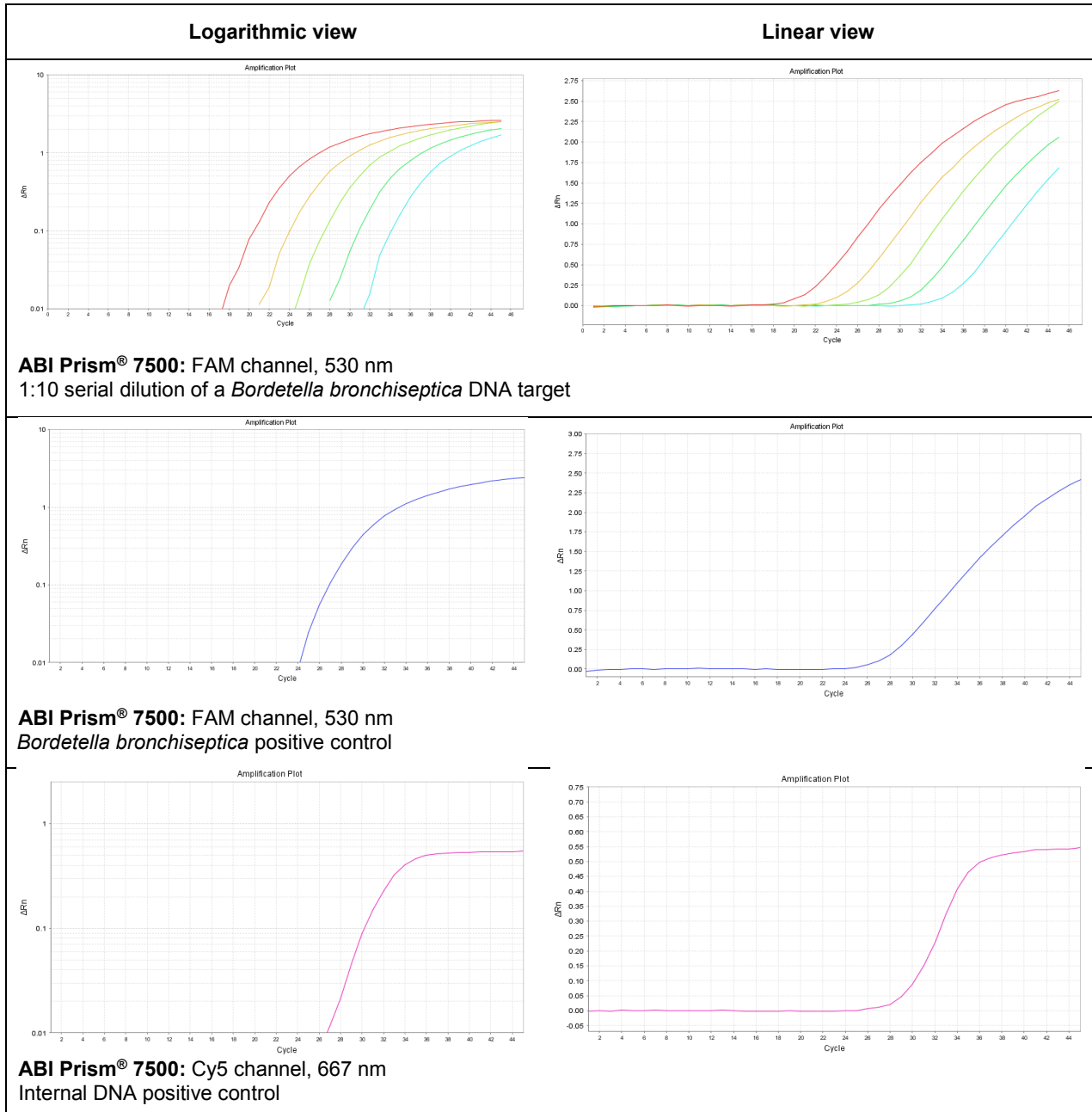
### 11.4. Signal in FAM channel in extraction negative control

- A contamination occurred during extraction.  
→ Repeat extraction and PCR using new reagents.  
→ Make sure that work space and instruments are cleaned at regular intervals.

## 12. Specifications and performance evaluation

### 12.1. Kit performance

Performance of BactoReal® Kit *Bordetella bronchiseptica* with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figure 1.



**Figure 1** Performance of BactoReal® Kit *Bordetella bronchiseptica*



## 12.2. Analytical sensitivity and linearity

BactoReal® Kit *Bordetella bronchiseptica* was tested with a 10-fold dilution series of a plasmid containing a fragment of *Bordetella bronchiseptica* DNA. At least 1 target copies/reaction could be detected. The limit of detection (LoD95 = smallest number of copies of target DNA which can be detected in 95% of cases) is 16 target copies/reaction and was determined by several replicates around the detection limit.

The assay shows **linearity** over the range of 100 to 1,000,000 target copies/reaction with a slope of 3.4 and a  $R_2$  of > 0.99 as shown in Figure 2.

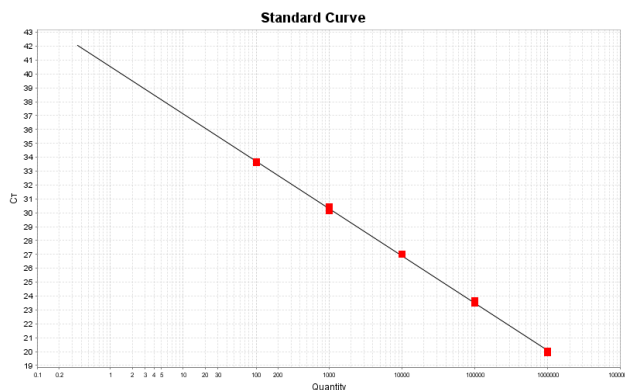


Figure 2 Ten-fold dilution series of a *B. bronchiseptica* DNA standard plotted against CT

## 12.3. Analytical specificity

The selection of highly specific primers and probes ensures analytical specificity. Primers and probes have been checked for possible homologies to currently published sequences by sequence comparison analysis. This validated the detection of so far known *B. bronchiseptica* strains. BactoReal® Kit *Bordetella bronchiseptica* was tested on 11 *B. bronchiseptica* isolates and on several different bacterial isolates (*Bordetella holmesii*, *Bordetella parapertussis*, *Bordetella pertussis*, *Escherichia coli*, *Mycoplasma gallisepticum*, *Mycoplasma hyopneumoniae* and *Mycoplasma synoviae*). No cross reactions were observed.

## 13. References

Rampelotto, R.F., Hörner, A., Hörner, C., Righi, R., Hörner, R. 2016. Pneumonia caused by *Bordetella bronchiseptica* in two HIV-positive patients. Sao Paulo Med J. 134(3):268-72