

# BactoReal<sup>®</sup> Kit

## *Ornithobacterium rhinotracheale*

### Manual

For use with the

- ABI<sup>®</sup> 7500 (Fast)
- Mx3005P<sup>®</sup>
- LightCycler<sup>®</sup> 480



For veterinary use only

<b>REF</b>	<b>DVEB06611, DVEB06613</b>		<b>100</b>
<b>REF</b>	<b>DVEB06651, DVEB06653</b>		<b>50</b>



**ingenetix GmbH**

Arsenalstraße 11  
 1030 Vienna, Austria  
 T +43(0)1 36 1980 198  
 F +43(0)1 36 1980 199  
 office@ingenetix.com  
 www.ingenetix.com

## Index

1. Product description .....	3
2. Pathogen information .....	3
3. Principle of real-time PCR .....	3
4. General Precautions .....	3
5. Contents of the Kit.....	4
5.1. BactoReal® Kit <i>Ornithobacterium rhinotracheale</i> order no. DVEB06611 or DVEB06651 .....	4
5.2. BactoReal® Kit <i>Ornithobacterium rhinotracheale</i> order no. DVEB06613 or DVEB06653 .....	4
6. Additionally required materials and devices.....	4
7. Preparation of real-time PCR .....	5
7.1. Pipetting scheme .....	5
7.2. Programming of the temperature profile .....	5
8. Interpretation of PCR-data .....	6
9. Troubleshooting.....	8
10. Specifications .....	8
10.1. Analytical sensitivity, LOD and linearity .....	8
10.2. Analytical specificity .....	9
11. Annex – symbols.....	9

## 1. Product description

BactoReal® Kit *Ornithobacterium rhinotracheale* is a real-time PCR assay for detection of *Ornithobacterium rhinotracheale* (ORT) DNA. This test was developed and validated for the ABI® 7500 (Fast) instrument (Thermo Fisher Scientific), LightCycler® 480 (Roche), and for Mx3005P® (Agilent) but is also suitable for other real-time PCR instruments. This test allows the rapid and sensitive detection of DNA of *Ornithobacterium rhinotracheale* from samples purified from tissues (e.g. with the QIAamp DNA Mini Kit).

BactoReal® Kit *Ornithobacterium rhinotracheale* detects the 16S rRNA gene of *Ornithobacterium rhinotracheale*. A probe-specific amplification-curve at 530 nm (FAM channel) indicates the amplification of *Ornithobacterium rhinotracheale* specific DNA.

An internal positive control system for detection in VIC/HEX channel, (554 nm, order no. DVEB06611 or DVEB06651) or Cy5 channel (667 nm; order no. DVEB06613 or DVEB06653) excludes false-negative interpretation of results due to inhibition of real-time PCR (see 8. Interpretation of PCR-data).

When using PCR-platforms not validated by ingenetix, an evaluation of the multiplex-PCR is recommended. Please be aware that some PCR-platforms have to be calibrated with the corresponding dye before performing multiplex-PCR.

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

## 2. Pathogen information

*Ornithobacterium rhinotracheale* (ORT) is a gram-negative bacterium causing respiratory disease in turkeys, chickens and other avian species. Clinical signs are nasal discharge, sneezing, coughing and sinusitis. *Ornithobacterium rhinotracheale* is currently the only species within the *Ornithobacterium* genus and is found worldwide.

## 3. Principle of real-time PCR

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

## 4. General Precautions

The user should always pay attention to the following:

- Always include a negative control per PCR-run (water instead of sample).
- Optional: for valid interpretation of results, a negative control should be included during DNA-extraction (for example extraction of water instead of sample material), in order to exclude false-positive results due to contamination with *Ornithobacterium rhinotracheale* DNA during extraction.
- Be careful when handling the positive control.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated workspace.
- Periodically decontaminate benches and devices.
- Use sterile pipette tips with filters.
- Thaw all components thoroughly at room temperature before starting an assay. When thawed, mix the components and centrifuge briefly.
- For MSDS, see [www.ingenetix.com](http://www.ingenetix.com).

## 5. Contents of the Kit

### 5.1. BactoReal® Kit *Ornithobacterium rhinotracheale* order no. DVEB06611 or DVEB06651

Labelling	Content	Amount		Storage
		DVEB06611	DVEB06651	
<i>Ornithobacterium rhinotracheale</i> Assay Mix (green cap)	Primer and probe (FAM) for detection of <i>O. rhinotracheale</i>	2 x 50 µl	1 x 50 µl	-15°C to -25°C
CR-1 Assay Mix (yellow cap)	Primer, probe (VIC/HEX) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-15°C to -25°C
<i>Ornithobacterium rhinotracheale</i> Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-15°C to -25°C
DNA Reaction Mix (white cap) <sup>#</sup>	Reaction Mix	2 x 500 µl	1 x 500 µl	<b>-20°C until first use, then at +4°C</b>
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-20°C to +4°C

<sup>#</sup>DNA Reaction Mix contains uracil-N glycosylase (UNG)

### 5.2. BactoReal® Kit *Ornithobacterium rhinotracheale* order no. DVEB06613 or DVEB06653

Labelling	Content	Amount		Storage
		DVEB06613	DVEB06653	
<i>Ornithobacterium rhinotracheale</i> Assay Mix (green cap)	Primer and probe (FAM) for detection of <i>O. rhinotracheale</i>	2 x 50 µl	1 x 50 µl	-15°C to -25°C
CR-3 Assay Mix (yellow cap)	Primer, probe (Cy5) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-15°C to -25°C
<i>Ornithobacterium rhinotracheale</i> Positive Control (red cap)	Control-DNA (approx. 10,000 target copies/µl)	1 x 25 µl	1 x 25 µl	-15°C to -25°C
DNA Reaction Mix (white cap) <sup>#</sup>	Reaction Mix	2 x 500 µl	1 x 500 µl	<b>-20°C until first use, then at +4°C</b>
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-20°C to +4°C

<sup>#</sup>DNA Reaction Mix contains uracil-N glycosylase (UNG)

The components of BactoReal® Kit *Ornithobacterium rhinotracheale* are stable until the expiry date stated on the label. Repeated thawing and freezing should be avoided. Please protect kit components from light.

## 6. Additionally required materials and devices

- Reagents and devices for DNA-extraction
- PCR-grade water
- 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 VIC/HEX or Cy5 channel
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material

## 7. Preparation of real-time PCR

Please make sure that at least one negative control (water, blue cap), as well as one positive control (red cap) and one extraction negative control (optional, recommended) are included per PCR run. Ingenetix highly recommends performing PCR analyses in duplicates, which increases the probability of detection of the pathogen and facilitates interpretation of results.

### 7.1. Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Water*	3.0 µl
	DNA Reaction Mix (2x)	10.0 µl
	<i>Ornithobacterium rhinotracheale</i> Assay Mix	1.0 µl
	CR Assay Mix	1.0 µl
	<b>Total volume Master Mix</b>	<b>15.0 µl</b>
Preparation of PCR assay	Master mix	15.0 µl
	Sample*	5.0 µl
	<b>Total volume</b>	<b>20.0 µl</b>

\*1-8 µl of the sample can be used. When using an amount other than 5 µl of the sample, the amount of H<sub>2</sub>O has to be changed accordingly.

**Positive Control:** As positive control please use 1 µl of the *Ornithobacterium rhinotracheale* Positive Control + 4 µl H<sub>2</sub>O.

Optional: a 1:10 dilution of the positive control can be used and defined as second standard value (approx. 1000 target copies/µl).

### 7.2. Programming of the temperature profile

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

**Select dyes:** FAM-TAMRA for detection of *Ornithobacterium rhinotracheale*  
Cy5-NONE (CR-3 Assay Mix) **or** VIC-TAMRA (CR-1 Assay Mix) for detection of IPC

**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	95°C 20 sec	95°C 5 sec
2 min*		60°C 1 min

For ABI® 7500:  
Ramp speed: Without “fast cycling” parameter

For LightCycler® 480 instrument:  
Detection format: 2 Color Hydrolysis Probe  
(dyes see above)

**\*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®, ParoReal and ViroReal® kits for the detection of DNA or RNA.

## 8. Interpretation of PCR-data

Examples for interpretation of positive reactions are shown in the amplification plots below.

**For a valid interpretation, the following criteria must be fulfilled:**

	<b>Ct/Cp (FAM channel) <i>O. rhinotracheale</i> target</b>	<b>Ct/Cp IPC target</b>	<b>Interpretation</b>
Negative control	Negative	36.0 ± 2	Valid
Positive control (undiluted, 1 µl/PCR)	28.0-31.0	36.0 ± 2	Valid
Or: positive control (1:10 diluted, 1 µl/PCR)	31.0-34.0	36.0 ± 2	Valid
Extraction negative control (optional)	Negative	36.0 ± 2	Valid
Negative sample	Negative	36.0 ± 2	Valid
Positive sample	Positive	Positive/Negative	Valid

**For analysis of PCR data please proceed as follows:**

For analysis of PCR results gained with BactoReal® Kit *Ornithobacterium rhinotracheale* please select fluorescence display options FAM channel for the *Ornithobacterium rhinotracheale* target and VIC/HEX channel (order no. DVEB06611, DVEB06651) or Cy5 channel (order no. DVEB06613, DVEB06653) for the internal positive control target. Samples with a positive Cp or Ct-value are considered positive. Please also check the presence of amplification-curves manually.

**Once the analysis is completed, the following results are possible:**

### 1. Signal in FAM channel:

→ DNA of *Ornithobacterium rhinotracheale* was amplified. The sample has to be interpreted as positive. *Ornithobacterium rhinotracheale* DNA can lead to a reduced or absent fluorescence signal of the internal positive control (competition of PCR).

### 2. No signal in FAM channel:

→ No *Ornithobacterium rhinotracheale* DNA is detectable in the sample. The sample has to be interpreted as negative.  
An inhibition of PCR cannot be excluded.

### 2a. No signal in FAM channel but signal of the internal positive control:

→ No *Ornithobacterium rhinotracheale* DNA is detectable in the sample. The sample has to be interpreted as negative.  
The positive signal of the internal positive control assay excludes a putative PCR inhibition.

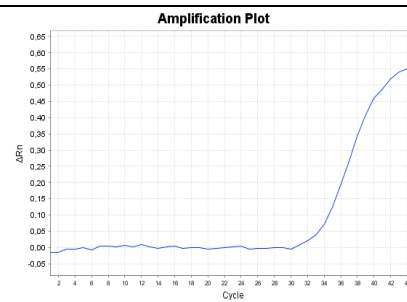
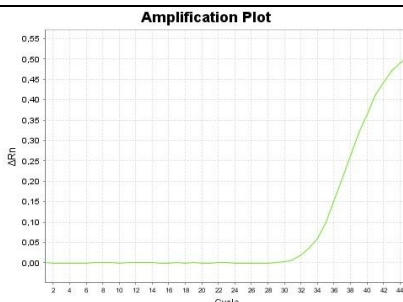
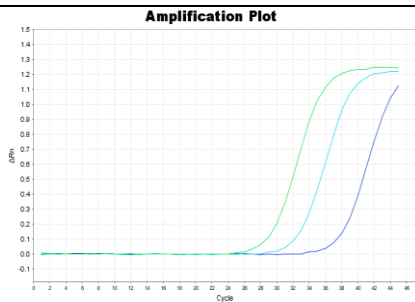
### 2b. No signals in FAM channel and no signal with internal positive control:

→ No interpretation statement can be made.  
Information about possible sources of error and their solution can be found in 9. Troubleshooting.

Detection of *Ornithobacterium rhinotracheale*

Detection of internal positive control CR-3

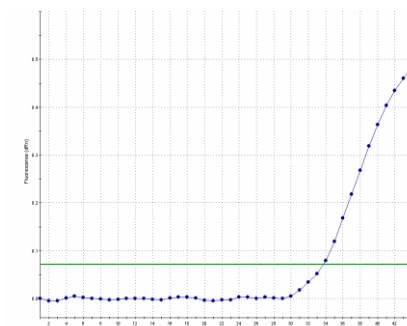
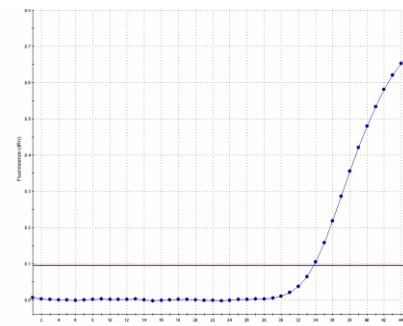
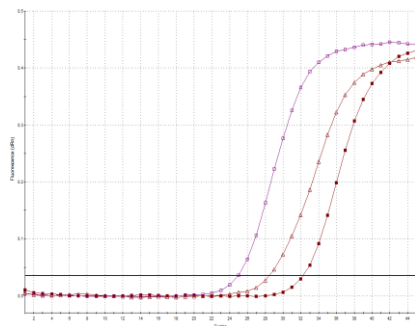
Detection of internal positive control CR-1



**ABI® 7500:** FAM channel, 530 nm  
1:10 serial dilution of *O. rhinotracheale* DNA

**ABI® 7500:** Cy5 channel, 667 nm  
Internal positive control

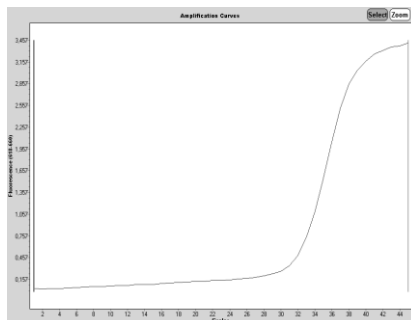
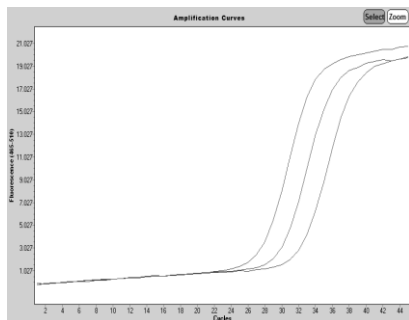
**ABI® 7500:** VIC channel, 554 nm  
Internal positive control



**Mx3005P®:** FAM channel  
1:10 serial dilution of *O. rhinotracheale* DNA

**Mx3005P®:** CY5 channel  
Internal positive control

**Mx3005P®:** HEX channel  
Internal positive control



**LightCycler® 480:** FAM channel  
1:10 serial dilution of *O. rhinotracheale* DNA

**LightCycler® 480:** Cy5 channel  
Internal positive control

## 9. Troubleshooting

### 1. No *Ornithobacterium rhinotracheale* specific signal with positive control:

- Incorrect programming of the temperature profile of the real-time PCR instrument.  
→ Compare the temperature profile with the protocol (see 7. Preparation of real-time PCR).
- Incorrect configuration of the PCR reaction.  
→ Check your work steps (see 7. Preparation of real-time PCR) and repeat the PCR, if necessary.

### 2. No signal with the internal positive control and no *Ornithobacterium rhinotracheale* specific signal with the sample:

- The PCR reaction was inhibited. No interpretation can be made.  
→ Make sure that you use a recommended method for DNA isolation and stick closely to the manufacturer's instructions.  
→ If no operating mistakes during extractions can be retraced, it is recommended to repeat the PCR with lower amounts of DNA-eluate (1/5 or 1/10 of sample volume + the adequate amount of H<sub>2</sub>O).
- Incorrect PCR conditions.  
→ Check the PCR conditions and repeat the PCR, if necessary.

### 3. *Ornithobacterium rhinotracheale* specific signal with the negative control:

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

### 4. *Ornithobacterium rhinotracheale* specific signal with the negative control of DNA-extraction:

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

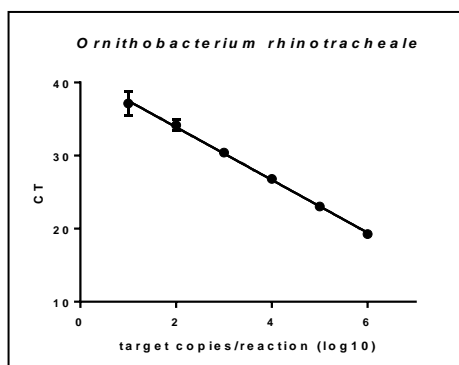
## 10. Specifications

BactoReal® Kit *Ornithobacterium rhinotracheale* was evaluated with the ABI® 7500 (Fast) instrument (Thermo Fisher Scientific), with the LightCycler® 480 (Roche) and the Mx3005P® (Agilent).

### 10.1. Analytical sensitivity, LOD and linearity

BactoReal® Kit *Ornithobacterium rhinotracheale* has been tested with a 10-fold dilution series of a plasmid containing a fragment of the 16S RNA gene of *Ornithobacterium rhinotracheale*. The analytical sensitivity is 1 target copies/PCR reaction. The limit of detection (LoD95 = smallest number of copies of target DNA which can be detected in 95% of cases) was determined by several replicates around the detection limit. The LOD is 5 target copies/reaction.

The assay shows **linearity** over the range of 10 to 1,000,000 target copies/reaction with a slope of -3.6 and a R<sup>2</sup> of 0.9874 as shown in Figure 1.



**Figure 1** Ten-fold dilution series of an *Ornithobacterium rhinotracheale* DNA standard plotted against Ct



## 10.2. Analytical specificity

The specificity is ensured by the selection of highly specific primers and probes. The primers and probes were checked for possible homologies to currently published sequences by sequence comparison analyses. This also validated the detection of so far known *Ornithobacterium rhinotracheale* strains. The kit is specific for *Ornithobacterium rhinotracheale*.

## 11. Annex – symbols



Batch code



Catalogue number



Contains sufficient for <n> tests



Use by



Manufactured by



Store at