BactoReal® Kit
Histophilus somni

Manual

For veterinary use only

REF **DVEB06111, DVEB06113** Σ **100**

REF **DVEB06151, DVEB06153** Σ **50**

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Explanation of symbols

LOT	Batch code		Use by
REF	Catalogue number		Manufactured by
Σ	Contains sufficient for <n> tests		Store at

1. Intended use

BactoReal® Kit *Histophilus somni* is a real-time PCR kit for the detection of *Histophilus somni.*

2. Product description

BactoReal® Kit *Histophilus somni* contains primers and probe for the amplification and detection of the 16S rRNA gene of *Histophilus somni*, a positive control, the amplification mix and an internal positive control. This test has been developed for use with the Applied Biosystems® (ABI) 7500 instrument (Thermo Fisher Scientific), LightCycler® 480 II (Roche) and Mx3005P® (Agilent), but is designed for compatibility with most real-time PCR instruments except capillary instruments. The kit allows rapid and sensitive detection of DNA of *Histophilus somni* in samples purified from reproductive tract, tracheal swabs, washes or from lung tissue (e.g. with the QIAamp® DNA Mini Kit, Qiagen).

BactoReal® Kit *Histophilus somni* detects the 16S rRNA gene of *Histophilus somni*. A probe-specific amplification-curve at 530 nm (FAM channel) indicates the amplification of *Histophilus somni*-specific DNA. The kit contains an internal positive control system (IPC) for detection in VIC channel, (554 nm, order no. DVEB06111 or DVEB06151) or Cy5 channel (667 nm; order no. DVEB06113 or DVEB06153) excluding false-negative results due to inhibition of real-time PCR.

When using PCR-platforms not tested by ingenetix, an evaluation of the multiplex-PCR is recommended. Keep in mind that some PCR-platforms first have to be calibrated with the corresponding dye before performing multiplex-PCR.

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

3. Pathogen information

Histophilus somni is an unencapsulated gram-negative bacterium and a member of the Pasteurellaceae family. It is a common commensal in the lower reproductive and upper respiratory tract of cattle and other ruminants. *H. somni* is one of the bacterial agents responsible for bovine respiratory disease (BRD) complex and associated with respiratory disease, arthritis, mastitis, myocarditis and reproductive failure in sheep and cattle. Other important bacterial pathogens of BRD are *Pasteurella multocida, Mannheimia haemolytica, Trueperella pyogenes* and *Mycoplasma bovis*. Mixed infections with these organisms do occur.

References:

Griffin D. 2010. Bovine pasteurellosis and other bacterial infections of the respiratory tract. Vet Clin North Am Food Anim Pract. 26(1):57-71

4. General Precautions

The user should 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 is included during DNA-extraction (for example extraction of water instead of sample material), in order to exclude false-positive results due to contamination with *Histophilus somni* 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 aerosol barrier pipette tips.
- Thaw all components thoroughly at room temperature before pipetting. When thawed, mix the components and centrifuge briefly.
- For MSDS, see www.ingenetix.com.

5. Additionally required materials and devices

- Aerosol barrier pipette tips
- Appropriate optical 96-well reaction plates or reaction tubes with optical closing material recommended by the manufacturer of the real-time PCR instrument
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM and VIC or Cy5 channel

Examples of real-time PCR instruments with required dye channels:

Instruments with **FAM** (510 nm) and **Cy5** (667 nm) channels:

e.g. ABI® 7500, QuantStudio™ 5 or 6 with the correct color calibration (Thermo Fisher Scientific), Mx3005P® (Agilent), LightCycler® 480 I, II or Cobas z 480 (Roche), Rotor-Gene Q 5plex (QIAGEN), CFX96 (BioRad), MIC (Corbett) or qTOWER with module 1&5 (Analytik Jena)

Instruments with **FAM** (510 nm) and **VIC** (554 nm) channels:

e.g. ABI® 7500, QuantStudio™ 5 or 6, ABI® 7000, StepOne Plus (Thermo Fisher Scientific), Mx3005P® (Agilent), LightCycler® 480 II or Cobas z 480 (Roche), Rotor-Gene Q 5plex (QIAGEN), CFX96 (BioRad) or qTOWER with module 1&2 (Analytik Jena)

Instruments with **FAM** (510 nm), **VIC** (554 nm) and **Cy5** (667 nm) channels:

e.g. ABI® 7500, QuantStudio™ 5 or 6 with the correct color calibration (Thermo Fisher Scientific), Mx3005P® (Agilent), LightCycler® 480 II or Cobas z 480 (Roche), Rotor-Gene Q 5plex (QIAGEN), CFX96 (BioRad) or qTOWER module 1&2&5 (Analytik Jena)

6. Contents of the Kit

6.1. Order Numbers of BactoReal® Kit *Histophilus somni*

Order Number	Reactions	IPC Assay	Dye Channel Pathogen	Dye Channel IPC	Target
DVEB06111	100	CR-1	FAM	VIC	16S rRNA gene
DVEB06151	50				
DVEB06113	100	CR-3		Cy5	
DVEB06153	50				

6.2 Content

Component	Content	Quantity		Storage
		100 rxn	50 rxn	
Histophilus somni Assay Mix (green cap)	Primer and probe (FAM) for detection of *Histophilus somni*	2 x 50 µl	1 x 50 µl	-15°C to -25°C
CR Assay Mix (yellow cap)	Primer, probe (VIC or Cy5) and target for detection of IPC	2 x 50 µl	1 x 50 µl	-15°C to -25°C
Histophilus somni 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)	Reaction Mix	2 x 500 µl	1 x 500 µl	-15°C to -25°C until first use, then at +4°C
Water (blue cap)	Water	1 x 1000 µl	1 x 1000 µl	-25°C to +4°C

The components of BactoReal® Kit *Histophilus somni* are stable until the expiry date stated on the label. Repeated freeze/thaw cycles should be avoided. Protect kit components from light.

DNA Reaction Mix: The Master Mix provided with the kit has been designed for reliable, high-sensitivity real-time PCR. The Master 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 RT-PCR inhibitors.

7. Preparation of real-time PCR

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 pathogen detection and facilitates interpretation of results.

7.1. Pipetting scheme

Sample: 1-8 µl sample can be used. When using a volume other than 5 µl, the volume of H_2O has to be adjusted accordingly.

Positive Control: Use 1 µl of *Histophilus somni* Positive Control + 4 µl H_2O.

		Per sample
Preparation of Master Mix (mix well)	Water	3.0 µl
	DNA Reaction Mix (2x)	10.0 µl
	Histophilus somni Assay Mix	1.0 µl
	CR Assay Mix	1.0 µl
	Total volume Master Mix	15.0 µl
Preparation of PCR assay	Master mix	15.0 µl
	Sample*	5.0 µl
	Total volume	20.0 µl

7.2. Programming of the temperature profile

Further information on programming the real-time PCR instrument can be found in the respective operator's manual. Keep in mind that some PCR-platforms first have to be calibrated with the corresponding dye before performing multiplex-PCR.

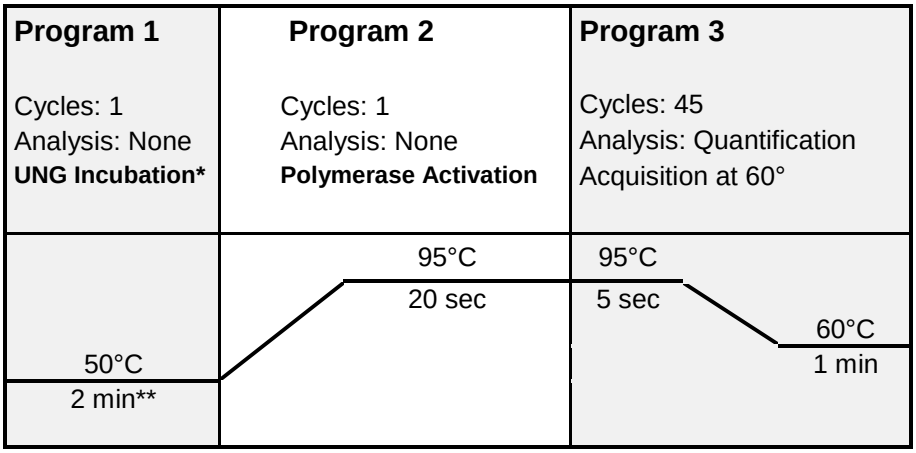
Select dyes: FAM-TAMRA for detection of *Histophilus somni*
VIC-TAMRA (CR-1 Assay Mix) **or** Cy5-NONE (CR-3 Assay Mix) for detection of IPC

Optionally, select reference dye (passive reference): ROX

Sample Volume: 20 µl

Temperature Profile:

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



For Applied Biosystems® 7500
Ramp speed: Without "fast cycling" parameter

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

*) UNG (Uracil-N-glycosylase) is a component of the DNA Reaction Mix as are dNTPs with dUTP to eliminate future amplicon carryover. Since the native template does not contain dUTP, it remains intact in the presence of the enzyme. The UNG enzyme eliminates contamination by any amplicon DNA that contains dUTP from a previous reaction by excising uracil residues from DNA cleaving the N-glycosylic bond creating abasic sites that do not serve as good DNA templates for Taq polymerase.

****Note:** If viral RNA is 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®, ViroReal®, MycoReal and ParoReal kits on all PCR instruments.

8. Interpretation of PCR-data

For analysis of PCR results gained with BactoReal® Kit *Histophilus somni* , select fluorescence display options FAM channel for the *Histophilus somni* target and VIC channel or Cy5 channel for the IPC. Samples with positive Cp or Ct-values are considered positive. Please, check amplification-curves and adjust the threshold manually, if necessary. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

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

	Ct/Cp (FAM channel) ***Histophilus somni*** **target**	**Ct/Cp[1)] IPC target**	**Interpretation**
Negative control	Negative	Positive	Valid
Positive control (undiluted, 1 µl/PCR)	Positive[1)]	Positive	Valid
Extraction negative control (optional)	Negative	Positive	Valid
Negative sample	Negative	Positive	Valid
Positive sample	Positive	Positive/Negative[2)]	Valid

1) The calculated Ct-value can vary depending on the real-time PCR instrument and software used. Ct-values of positive control and IPC are expected at ~ Ct 30.

   Always verify and compare IPC Ct-values with respect to shape and increment of the fluorescence reporter signal of the amplification curve to exclude inhibition of the reaction.

2) High pathogen load in the sample can lead to a reduced or absent fluorescence signal of the IPC.

8.1. Signal in FAM channel

→ DNA of *Histophilus somni* has been amplified. The sample has to be interpreted as positive.

8.2. No signal in FAM channel but signal with IPC

→ No *Histophilus somni* DNA is detectable in the sample. The sample has to be interpreted as negative. The positive signal of the IPC excludes a putative PCR inhibition.

8.3. No signals in FAM channel and no signal with IPC

→ No interpretation can be made.

Information about possible error sources and their solution can be found in 9. Troubleshooting.

9. Troubleshooting

9.1. No *Histophilus somni* 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.

9.2. No signal with IPC and no *Histophilus somni* specific signal with sample

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 volume of H_2O).

Incorrect PCR conditions.
→ Check the PCR conditions and repeat the PCR, if necessary.

9.3. *Histophilus somni* specific signal with 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 workspace and instruments are decontaminated at regular intervals.

9.4. *Histophilus somni* specific signal with negative control of extraction (optional)

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

10. Specifications and performance evaluation

BactoReal® Kit *Histophilus somni* has been evaluated with an Applied Biosystems® 7500 instrument (Thermo Fisher Scientific). For further validation data contact ingenetix GmbH.

10.1. Analytical sensitivity and linearity

BactoReal® Kit *Histophilus somni* has been tested with a 10-fold dilution series of a plasmid containing a fragment of *Histophilus somni* DNA. Analytical sensitivity is 10 target copies/PCR reaction. The limit of detection (LoD95 = smallest number of copies of target DNA which can be detected in 95% of cases) of 29 target copies/reaction has been 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 -3.3 and a R^2 of > 0.99 as shown in Figure 1.

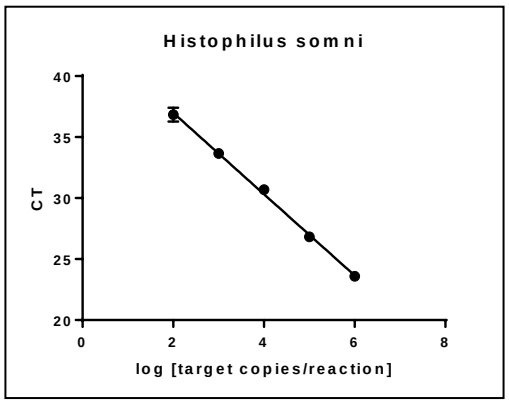


Figure 1 Ten-fold dilution series of a *Histophilus somni* DNA standard plotted against Ct

10.2. Analytical specificity

The specificity is ensured by the selection of highly specific primers and probes. Primers and probes have been checked for possible homologies to currently published sequences by sequence comparison analyses. This also validated the detection of so far known *Histophilus somni* strains.

10.3. Kit performance

Performance of BactoReal® Kit *Histophilus somni* with an ABI® 7500 instrument is shown in Figure 2.

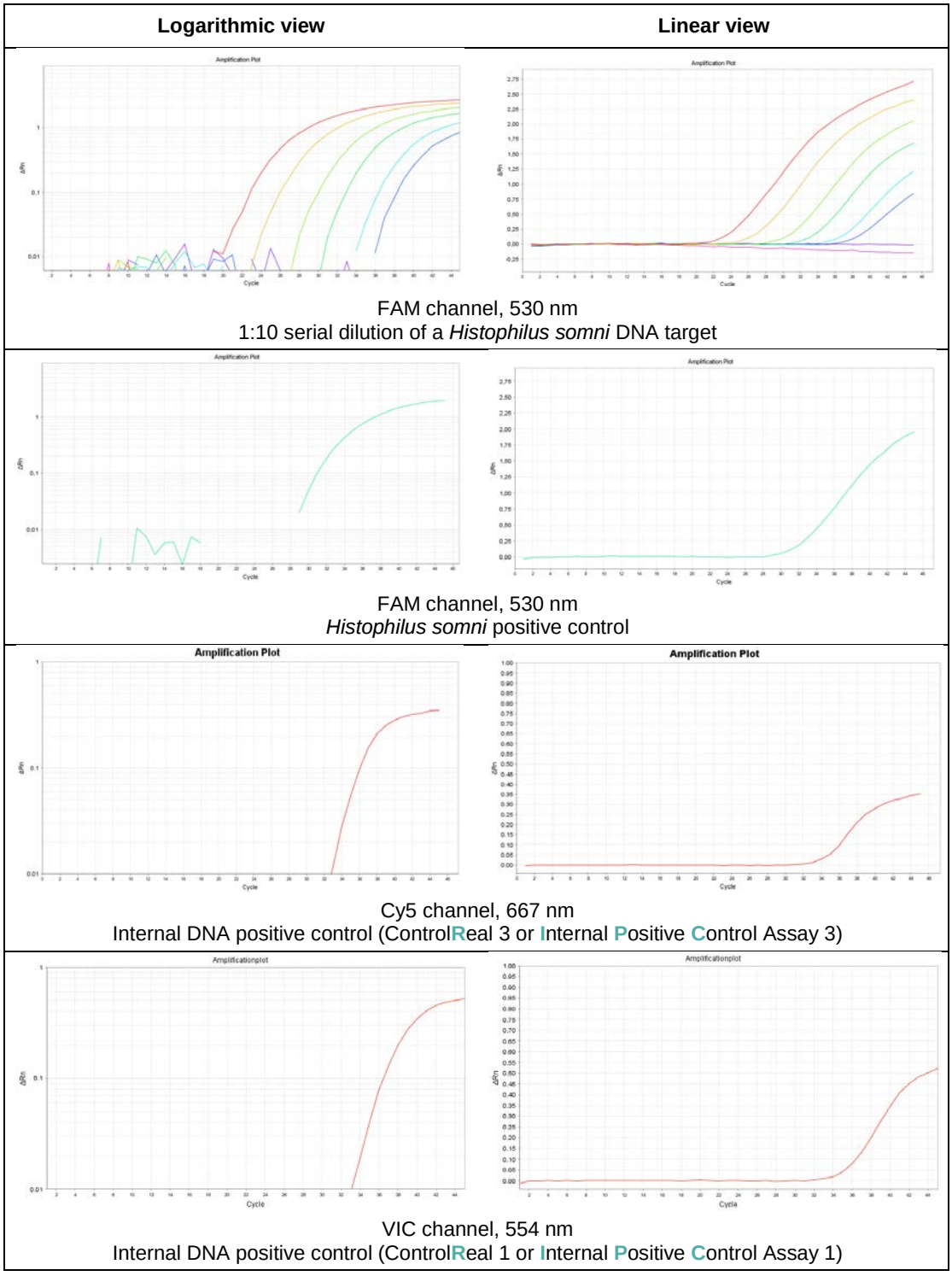


Figure 2 Performance of BactoReal® Kit *Histophilus somni*