Instructions for Use

RealStar®
Bordetella PCR Kit 1.0

01/2017  EN
RealStar®

Bordetella PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)
VERSANT® kPCR Molecular System AD (Siemens Healthcare)
ABI Prism® 7500 SDS (Applied Biosystems)
ABI Prism® 7500 Fast SDS (Applied Biosystems)
Rotor-Gene® 6000 (Corbett Research)
Rotor-Gene® Q5/6 plex Platform (QIAGEN)
CFX96™ Real-Time PCR Detection System (Bio-Rad)
CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
LightCycler® 480 Instrument II (Roche)
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1. Intended Use

The RealStar® Bordetella PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* specific DNA.

2. Kit Components

<table>
<thead>
<tr>
<th>Lid Color</th>
<th>Component</th>
<th>Number of Vials</th>
<th>Volume [µl/Vial]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>Master A</td>
<td>8</td>
<td>60</td>
</tr>
<tr>
<td>Purple</td>
<td>Master B</td>
<td>8</td>
<td>180</td>
</tr>
<tr>
<td>Green</td>
<td>Internal Control</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Red</td>
<td>Positive Control</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>White</td>
<td>Water (PCR grade)</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>

3. Storage

- The RealStar® Bordetella PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

**NOTE**

- Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer’s instructions and recommendations.
- It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).
5. Background Information

*Bordetella pertussis* and *Bordetella parapertussis* are the causative pathogens of whooping cough, a highly contagious, acute coughing illness in human [1, 2]. Other species of the genus *Bordetella* can also cause respiratory disease in humans. *Bordetella holmesii* was most recently associated with pertussis-like illness [3, 4] and *Bordetella bronchiseptica* infects a broad range of mammals, including humans, occasionally causing cough illnesses. Severe infections can occur in persons who are immunocompromised [5].

All *Bordetella* species that cause respiratory disease in humans carry transposable DNA elements, the so called insertion sequences (IS). These insertion sequences are usually present in multiple copies per genome (see Table 1), allowing the design of PCR systems that display a high sensitivity.

**Table 1: Bordetella insertion sequences IS481 and IS1001, adapted from Loeffelholz [6]**

<table>
<thead>
<tr>
<th>Insertion Sequence</th>
<th><em>B. pertussis</em></th>
<th><em>B. parapertussis</em></th>
<th><em>B. holmesii</em></th>
<th><em>B. bronchiseptica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481</td>
<td>+/&gt;50</td>
<td>-/NA</td>
<td>+/8-10</td>
<td>(+)/ND</td>
</tr>
<tr>
<td>IS1001</td>
<td>-/NA</td>
<td>+/-20</td>
<td>-/NA</td>
<td>(+)/1-7</td>
</tr>
</tbody>
</table>

1 Symbols and abbreviations: +, present in all isolates; (+), present in some isolates; -, absent from all isolates; NA, not applicable; ND, not determined.

2 Human-derived *B. bronchiseptica* isolates only.

3 One of 73 human-derived isolates was positive.

4 Four of 73 human-derived isolates were positive.

With more than 50 copies per genome [7], the insertion sequence IS481 is the favourable target for the detection of *Bordetella pertussis*. This target is also present in *Bordetella holmesii*, with copy numbers ranging from 8 to 10 copies per genome [7] and is found infrequently in *Bordetella bronchiseptica* strains [8].

The genome of *Bordetella parapertussis* carries approximately 20 copies of the insertion sequence IS1001, which facilitates a highly sensitive PCR detection, but is also found in some *Bordetella bronchiseptica* strains with copy numbers ranging from 1 to 7 copies per genome [7].

There are differences in the diagnostic needs of clinical versus public health settings. In the clinical setting, the goal is to optimise sensitivity (not to miss cases) while providing rapid results. This ensures appropriate treatment and prevents further transmission. In the public health setting, a high degree of specificity (in most countries a *B. pertussis* infection is reportable, but not an infection with other *Bordetella* species) is needed to avoid unnecessary and ineffective public health interventions [9].

In favour of highest sensitivity while dispensing highest specificity, the RealStar® Bordetella PCR Kit 1.0 targets the IS481 for the detection of *Bordetella pertussis* and the IS1001 for the detection of *Bordetella parapertussis*. 
6. Product Description

The RealStar® Bordetella PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* specific DNA. The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for *Bordetella pertussis* (Target IS481) DNA are labelled with the fluorophore FAM™ whereas the probes specific for *Bordetella parapertussis* (Target IS1001) DNA are labelled with a fluorophore showing similar characteristics to Cy®5. The probe specific for Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of *Bordetella pertussis* (Target IS481) and *Bordetella parapertussis* (Target IS1001) specific DNA as well as the detection of the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes
The RealStar® Bordetella PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Positive Control [Bordetella pertussis + Bordetella parapertussis]
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and target detection of *Bordetella pertussis* (Target IS481) and *Bordetella parapertussis* (Target IS1001) specific DNA and Internal Control in one reaction setup.

### 6.1 Real-Time PCR Instruments

The RealStar® Bordetella PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

### 7. Warnings and Precautions

*Read the Instructions for Use carefully before using the product.*

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
• Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
• Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
• Do not use components of the kit that have passed their expiration date.
• Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted DNA is the starting material for the RealStar® Bordetella PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® DNA Mini Kit (QIAGEN)
- QIAasymp®ony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® Bordetella PCR Kit 1.0 has to be validated by the user.

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

**CAUTION**

If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Bordetella PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.
If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Internal Control</td>
<td>1 µl</td>
<td>12 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>21 µl</td>
<td>252 µl</td>
</tr>
</tbody>
</table>

If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, the IC must not be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Volume Master Mix</td>
<td>20 µl</td>
<td>240 µl</td>
</tr>
</tbody>
</table>

8.3 Reaction Setup

Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the control (Positive or Negative Control).

Make sure that each Positive Control and at least one Negative Control is used per run.

Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.

Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).
9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® Bordetella PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

<table>
<thead>
<tr>
<th>Settings</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Volume</td>
<td>30 µl</td>
</tr>
<tr>
<td>Ramp Rate</td>
<td>Default</td>
</tr>
<tr>
<td>Passive Reference</td>
<td>ROX™</td>
</tr>
</tbody>
</table>

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

<table>
<thead>
<tr>
<th>Target</th>
<th>Detector Name</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bordetella pertussis</em> specific DNA</td>
<td>Target IS481</td>
<td>FAM™</td>
<td>(None)</td>
</tr>
<tr>
<td><em>Bordetella parapertussis</em> specific DNA</td>
<td>Target IS1001</td>
<td>Cy5®</td>
<td>(None)</td>
</tr>
<tr>
<td>Internal Control</td>
<td>IC</td>
<td>JOE™</td>
<td>(None)</td>
</tr>
</tbody>
</table>

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

<table>
<thead>
<tr>
<th></th>
<th>Analysis Mode</th>
<th>Cycle Repeats</th>
<th>Acquisition</th>
<th>Temperature [°C]</th>
<th>Time [min:sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>Hold</td>
<td>1</td>
<td></td>
<td>95</td>
<td>02:00</td>
</tr>
<tr>
<td>Amplification</td>
<td>Cycling</td>
<td>45</td>
<td>yes</td>
<td>58</td>
<td>00:45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>00:15</td>
</tr>
</tbody>
</table>
10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Bordetella PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

For a valid diagnostic test run, the following control conditions must be met:

<table>
<thead>
<tr>
<th>Control ID</th>
<th>Detection Channel</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control [Bordetella pertussis + Bordetella parapertussis]</td>
<td>FAM™</td>
<td>Cy®5</td>
</tr>
</tbody>
</table>
|                            | +     | +     | +/−* | *Bordetella pertussis and Bordetella parapertussis specific DNA detected.  
| Negative Control           | −     | −     | +    | *Bordetella parapertussis specific DNA detected.  

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A diagnostic test run is invalid, (i) if the run has not been completed or (ii) if any of the control conditions for a valid diagnostic test run are not met.

In case of an invalid diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

<table>
<thead>
<tr>
<th>Detection Channel</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM™</td>
<td>Cy®5</td>
</tr>
</tbody>
</table>
| +     | +     | +/−* | *Bordetella pertussis and Bordetella parapertussis specific DNA detected.  
| +     | −     | +/−* | *Bordetella pertussis specific DNA detected.  
| −     | +     | +/−* | *Bordetella parapertussis specific DNA detected.  
| −     | −     | +    | Neither Bordetella pertussis nor Bordetella parapertussis specific DNA detected. The sample does not contain detectable amounts of Bordetella pertussis or Bordetella parapertussis specific DNA.  
| −     | −     | −    | PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.  

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results either in the FAM™ detection channel or in the Cy®5 detection channel. High Bordetella pertussis (Target IS481) and/or Bordetella parapertussis (Target IS1001) DNA load/s in the sample can lead to reduced or absent Internal Control signals.

1 A positive signal in the FAM™ channel could also be due to the presence of Bordetella holmesii or B. bronchiseptica DNA in the sample.

2 A positive signal in the Cy®5 channel could also be due to the presence of Bordetella bronchiseptica DNA in the sample.
11. Performance Evaluation

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Bordetella PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of *Bordetella pertussis* (Target IS481) or *Bordetella parapertussis* (Target IS1001) specific DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified *Bordetella pertussis* (Target IS481) DNA and *Bordetella parapertussis* (Target IS1001) DNA.

Table 2: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *Bordetella pertussis* (Target IS481) specific DNA

<table>
<thead>
<tr>
<th>Input Conc. [copies/µl]</th>
<th>Number of Replicates</th>
<th>Number of Positives</th>
<th>Hit Rate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.600</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>10.000</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>3.160</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>1.000</td>
<td>18</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>0.316</td>
<td>18</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>0.100</td>
<td>18</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>0.032</td>
<td>18</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>0.010</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.003</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The analytical sensitivity of the RealStar® Bordetella PCR Kit 1.0 was determined by Probit analysis:

- For the detection of *Bordetella pertussis* (Target IS481) specific DNA, the analytical sensitivity is 0.74 copies/µl for [95% confidence interval 0.39 to 2.08 copies/µl]
- For the detection of *Bordetella parapertussis* (Target IS1001) specific DNA, the analytical sensitivity is 0.60 copies/µl for [95% confidence interval 0.35 to 1.54 copies/µl]
11.2 Analytical Specificity

The analytical specificity of the RealStar® Bordetella PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant Bordetella genotypes will be detected.

The analytical specificity of the RealStar® Bordetella PCR Kit 1.0 was evaluated by testing a panel of genomic RNA/DNA extracted from bacteria related to Bordetella pertussis and Bordetella parapertussis and other pathogens causing similar symptoms as Bordetella pertussis and Bordetella parapertussis.

The RealStar® Bordetella PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Human adenovirus 1
- Human adenovirus 4
- Enterovirus, Coxsackie A3
- Human metapneumovirus A2
- Human metapneumovirus B2
- Influenza A virus
- Influenza B virus
- Human parainfluenza virus 1
- Human parainfluenza virus 2
- Human parainfluenza virus 3
- Human parainfluenza virus 4a/b
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- Chlamydia pneumoniae
- Chlamydia psittaci
- Corynebacterium diphtheriae
- Haemophilus influenzae
- Legionella pneumophila
- Moraxella catarrhalis
- Mycobacterium avium
- Mycoplasma pneumoniae
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Staphylococcus aureus
- Streptococcus pneumoniae
- Streptococcus pyogenes
- Bordetella bronchiseptica IS481-
- Bordetella pertussis IS481
- Bordetella parapertussis IS1001

11.3 Precision

Precision of the RealStar® Bordetella PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analysis.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (Ct) - values. At least six replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

### Table 4: Precision data for the detection of Bordetella pertussis (Target IS481) and Bordetella parapertussis (Target IS1001) specific DNA

<table>
<thead>
<tr>
<th></th>
<th>Average Threshold Cycle (Ct)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-Assay Variability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target IS481</td>
<td>30.84</td>
<td>0.12</td>
<td>0.40</td>
</tr>
<tr>
<td>Target IS1001</td>
<td>30.44</td>
<td>0.14</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Inter-Assay Variability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target IS481</td>
<td>30.83</td>
<td>0.12</td>
<td>0.37</td>
</tr>
<tr>
<td>Target IS1001</td>
<td>30.63</td>
<td>0.20</td>
<td>0.65</td>
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<tr>
<td><strong>Inter-Lot Variability</strong></td>
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<tr>
<td>Target IS481</td>
<td>30.76</td>
<td>0.12</td>
<td>0.38</td>
</tr>
<tr>
<td>Target IS1001</td>
<td>30.45</td>
<td>0.10</td>
<td>0.34</td>
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<tr>
<td><strong>Total Variability</strong></td>
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<td></td>
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<tr>
<td>Target IS481</td>
<td>30.79</td>
<td>0.12</td>
<td>0.40</td>
</tr>
<tr>
<td>Target IS1001</td>
<td>30.56</td>
<td>0.20</td>
<td>0.65</td>
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</table>

### Table 5: Precision data for the detection of the Internal Control

<table>
<thead>
<tr>
<th></th>
<th>Average Threshold Cycle (Ct)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
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<tbody>
<tr>
<td><strong>Intra-Assay Variability</strong></td>
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<tr>
<td>Internal Control</td>
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<td><strong>Inter-Assay Variability</strong></td>
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<tr>
<td>Internal Control</td>
<td>26.71</td>
<td>0.16</td>
<td>0.61</td>
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<tr>
<td><strong>Inter-Lot Variability</strong></td>
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<tr>
<td>Internal Control</td>
<td>26.94</td>
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<td>0.63</td>
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<tr>
<td><strong>Total Variability</strong></td>
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<tr>
<td>Internal Control</td>
<td>26.82</td>
<td>0.23</td>
<td>0.84</td>
</tr>
</tbody>
</table>
12. Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the *Bordetella pertussis* and *Bordetella parapertussis* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® Bordetella PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.
- Many *Bordetella* species carry transposable DNA elements, the so called insertion sequences (IS). Notably IS481 is present with high copy numbers in the genome of *Bordetella pertussis* and IS1001 occurs in the genome of *Bordetella parapertussis*. The transposable element IS481 is also found with medium copy numbers in the genome of *Bordetella holmesii* and with a very low incidence in the genome of some strains of *Bordetella bronchiseptica*. The transposable element IS1001 can also be present in low copy numbers in the genome of *Bordetella bronchiseptica*.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® Bordetella PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

- e-mail: support@altona-diagnostics.com
- phone: +49-(0)40-5480676-0

15. Literature


16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gen®e, QIAamp®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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The RealStar® Bordetella PCR Kit 1.0 is a CE-marked diagnostic kit according to the European in vitro diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

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17. Explanation of Symbols

- IVD: In vitro diagnostic medical device
- LOT: Batch code
- CAP: Cap color
- REF: Product number
- CONT: Content
- NUM: Number
- COMP: Component
- GTIN: Global trade identification number
- Consult instructions for use
- Contains sufficient for “n” tests/reactions (rxns)
- Temperature limit
- Use-by date
- Manufacturer
- Caution
- Note
- Version