Bordetella pertussis IS481/ptxA

Insertion sequence IS481/Toxin subunit 1 precursor (ptxA) gene

150 tests
Introduction to Bordetella pertussis_IS481/ptxA

Bordetella pertussis is a Gram-negative bacterium of the genus Bordetella, and the causative agent of pertussis or whooping cough. Unlike B. bronchiseptica, B. pertussis is non-motile. There does not appear to be a zoonotic reservoir for B. pertussis, humans are its only host. The bacterium is spread by coughing and by nasal drops. The incubation period is 7-14 days. Whooping Cough, or Pertussis, is an infection of the respiratory system and characterized by a “whooping” sound when the person breathes in. In the US it killed 5,000 to 10,000 people per year before a vaccine was available. Vaccination has transformed this and between 1985-88 less than 100 children died from pertussis. Worldwide in 2000, according to the WHO, around 39 million people were infected annually and about 297,000 died.

Whooping Cough occurs most with children under the age of one when they are immunized or children with faded immunity, normally around the age 11 through 18. The signs and symptoms are similar to a common cold: runny nose, sneezing, mild cough, and low-grade fever. After a spell, they might make a “whooping” sound when breathing in or vomit. Adults have milder symptoms, like prolonged coughing without the “whoop.” Pertussis is highly contagious and may become airborne when the person coughs, sneezes, or laughs. People infected by this disease are more contagious in the earliest stages of it, normally 2 weeks after the coughing begins. Whooping Cough can be prevented by the Pertussis Vaccine which is part of the DTaP (diphtheria, tetanus, acellular Pertussis) immunization. The paroxysmal cough precedes a crowing inspiratory sound characteristic of pertussis. (Infants less than 6 months may not have the typical whoop.) A coughing spell may last a minute or more, producing cyanosis, apnoea and seizures. A prolonged cough may be irritating and sometimes a disabling cough may go undiagnosed in adults for many months.

Bordetella pertussis also produces a lymphocytosis-promoting factor, which causes a decrease in the entry of lymphocytes into lymph nodes. This can lead to a condition known as lymphocytosis, with a complete lymphocyte count over of 4000/μL in adults or over 8000/μL in children.

The Insertion sequence 481 (IS481) is a high copy number target that is present in the B. pertussis genome at 80 to 100 copies. The toxin subunit 1 precursor (ptxA) gene is a single copy number target that will detect the pertussis toxin that is produced by Bordetella pertussis, this toxin plays a central role in the pathogenesis of pertussis.
The PCR Max qPCR Kit for Bordetella pertussis IS481/ptxA (B.pertussis-IS481/ptxA) genomes is designed for the in vitro quantification of B.pertussis-IS481/ptxA genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the B.pertussis-IS481/ptxA genome.

The primers and probe sequences in this kit have 100% homology with a broad range of B.pertussis-IS481/ptxA sequences based on a comprehensive bioinformatics analysis.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to help@pcrmax.com and our bioinformatics team will answer your question.
Kit Contents

- **B.pertussis-IS481/ptxA specific primer/probe mix (150 reactions BROWN)**
  FAM labelled

- **B.pertussis-IS481/ptxA positive control template (for Standard curve RED)**

- **Internal extraction control primer/probe mix (150 reactions BROWN)**
  VIC labelled as standard

- **Internal extraction control DNA (150 reactions BLUE)**

- **Endogenous control primer/probe mix (150 reactions BROWN)**
  FAM labelled

- **RNAse/DNAsese free water (WHITE)**
  for resuspension of primer/probe mixes and internal extraction control DNA

- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and standard curve preparation

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Reagents and equipment to be supplied by the user

**Real-Time PCR Instrument**

**DNA extraction kit**
This kit designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

**Lyophilised 2x qPCR Mastermix**
This kit is designed to be compatible with all commercially available Mastermixes that run with standard cycling conditions.

**Pipettors and Tips**

**Vortex and centrifuge**

**Thin walled 1.5 ml PCR reaction tubes**
Kit storage and stability
This kit is stable at room temperature but should be stored at -20°C on arrival. Once the
lyophilised components have been resuspended they should not be exposed to
temperatures above -20°C for longer than 30 minutes and unnecessary repeated
freeze/thawing should be avoided. The kit is stable for six months from the date of
resuspension under these circumstances.
If a standard curve dilution series is prepared this can be stored frozen for an extended
period. If you see any degradation in this serial dilution a fresh standard curve can be
prepared from the positive control.
PCRmax does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material
All kinds of sample material suited for PCR amplification can be used. Please ensure the
samples are suitable in terms of purity, concentration, and DNA integrity (An internal PCR
control is supplied to test for non specific PCR inhibitors). Always run at least one negative
control with the samples. To prepare a negative-control, replace the template DNA sample
with RNAse/DNAse free water.

Dynamic range of test
Under optimal PCR conditions PCRmax B.pertussis-IS481/ptxA detection kits have very
high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers
This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug
purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the
USA or the appropriate regulatory authorities in the country of use. During the warranty period PCRmax detection kits allow
precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP
guidelines and the manufacturer’s recommendations the right to claim under guarantee is expired. PCR is a proprietary
technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have
been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from
Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by
contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied
Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the S’
nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.
S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The
Perkin-Elmer Corporation.

Trademarks
PCRmax™ is a trademark of PCRmax Ltd.
The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche
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the PCRmax reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by
Hoffmann-LaRoche Inc.
Principles of the test

**Real-time PCR**
A B. pertussis-IS481/ptxA specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the B. pertussis-IS481/ptxA DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms.

**Positive control**
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of B. pertussis-IS481/ptxA copy number / Cq value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target B. pertussis-IS481/ptxA gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

**Negative control**
To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNAse/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run.
Internal DNA extraction control
When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and real-time PCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

A separate primer and probe mix are supplied with this kit to detect the exogenous DNA using real-time PCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the B. pertussis-IS481/ptxA target DNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3.

Endogenous control
To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the B. pertussis-IS481/ptxA primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Carry-over prevention using UNG (optional)
Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial Mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. PCRmax recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step.
**Reconstitution Protocol**

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not split upon opening the tube.

2. **Reconstitute the kit components in the RNAse/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in water</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-PCR pack</td>
<td></td>
</tr>
<tr>
<td>B.pertussis-IS481/ptxA primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>165 µl</td>
</tr>
<tr>
<td>Pre-PCR heat-sealed foil</td>
<td></td>
</tr>
<tr>
<td>Internal extraction control DNA (BLUE)</td>
<td>600 µl</td>
</tr>
</tbody>
</table>

3. **Reconstitute the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in template preparation buffer</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-PCR heat-sealed foil</td>
<td></td>
</tr>
<tr>
<td>B.pertussis-IS481/ptxA Positive Control Template (RED)*</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

**DNA extraction**

The internal extraction control DNA can be added either to the DNA lysis/extraction buffer or to the DNA sample once it has been resuspended in lysis buffer.

**DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.**

1. **Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

2. **Complete DNA extraction according to the manufacturers protocols.**
Real-time PCR detection protocol

1. For each DNA sample prepare a reaction mix according to the table below:
   Include sufficient reactions for positive and negative controls.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilised 2x qPCR Mastermix</td>
<td>10 µl</td>
</tr>
<tr>
<td>B.pertussis-IS481/ptxA primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNAse/DNAse free water (WHITE)</td>
<td>3 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td><strong>15 µl</strong></td>
</tr>
</tbody>
</table>

2. For each DNA sample prepare an endogenous control reaction according to the table below (Optional):
   This control reaction will provide crucial information regarding the quality of the biological sample.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilised 2x qPCR Mastermix</td>
<td>10 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNAse/DNAse free water (WHITE)</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td><strong>15 µl</strong></td>
</tr>
</tbody>
</table>

3. Pipette 15µl of each mix into individual wells according to your real-time PCR experimental plate set up.

4. Prepare sample DNA templates for each of your samples.

5. Pipette 5µl of DNA template into each well, according to your experimental plate set up.
   For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl.

6. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilised 2x qPCR Mastermix</td>
<td>10 µl</td>
</tr>
<tr>
<td>B.pertussis-IS481/ptxA primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNAse/DNAse free water (WHITE)</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td><strong>15 µl</strong></td>
</tr>
</tbody>
</table>
7. **Preparation of standard curve dilution series.**

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

<table>
<thead>
<tr>
<th>Standard Curve</th>
<th>Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1 Positive control (RED)</td>
<td>$2 \times 10^5$ per µl</td>
</tr>
<tr>
<td>Tube 2</td>
<td>$2 \times 10^4$ per µl</td>
</tr>
<tr>
<td>Tube 3</td>
<td>$2 \times 10^3$ per µl</td>
</tr>
<tr>
<td>Tube 4</td>
<td>$2 \times 10^2$ per µl</td>
</tr>
<tr>
<td>Tube 5</td>
<td>20 per µl</td>
</tr>
<tr>
<td>Tube 6</td>
<td>2 per µl</td>
</tr>
</tbody>
</table>

8. **Pipette 5µl of standard template into each well for the standard curve according to your experimental plate set up.**
The final volume in each well is 20µl.

**Amplification Protocol**

Amplification conditions using Lyophilised 2x qPCR Mastermix.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG treatment (if required) **</td>
<td>15 mins</td>
<td>37 °C</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>2 mins</td>
<td>95 °C</td>
</tr>
<tr>
<td>Denaturation</td>
<td>10 secs</td>
<td>95 °C</td>
</tr>
<tr>
<td>DATA COLLECTION *</td>
<td>60 secs</td>
<td>60 °C</td>
</tr>
</tbody>
</table>

* Fluorogenic data should be collected during this step through the FAM and VIC channels
** Required if your Mastermix includes UNG to prevent PCR carryover contamination
## Interpretation of Results

<table>
<thead>
<tr>
<th>Target (FAM)</th>
<th>Internal control (VIC)</th>
<th>Positive control</th>
<th>Negative control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>+ / -</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUANTITATIVE RESULT calculate copy number</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUANTITATIVE RESULT calculate copy number</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>POSITIVE QUALITATIVE RESULT do not report copy number as this may be due to poor sample extraction</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NEGATIVE RESULT</td>
</tr>
</tbody>
</table>

| + / - | + / - | + | ≤ 35 | EXPERIMENT FAILED due to test contamination |
| + / - | + / - | + | > 35 | * |
| -     | -     | + | -    | SAMPLE PREPARATION FAILED |
| + / - | + / - | - | + / - | EXPERIMENT FAILED |

Positive control template (RED) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:

**SAMPLE POSITIVE**

If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.

**INCONCLUSIVE**

If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.
**Internal PCR control**

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a B. pertussis-IS481/ptxA sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

**Endogenous control**

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.