**INSTRUCTIONS FOR USE**

**VetMAX™ C. burnetii & Chlamydyphila spp. Kit**

**TaqMan™ real-time PCR for detecting *Coxiella burnetii* and *Chlamydophila* spp.**

**Catalog Number** TFQQCHP

**Doc. Part No.** 100020451 **Pub. No.** MAN0008883 **Rev.** E.0

<table>
<thead>
<tr>
<th>Technology</th>
<th>Species</th>
<th>Nucleic acid isolated from matrices</th>
<th>Test type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time PCR (DNA)</td>
<td>Bovine</td>
<td>Placenta, placental swab</td>
<td>Individual</td>
</tr>
<tr>
<td>- Triplex</td>
<td>Small ruminants (sheep, goat)</td>
<td>Vaginal and cervical swab</td>
<td></td>
</tr>
<tr>
<td>- Endogenous IPC</td>
<td></td>
<td>Fetuses, amniotic fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vaginal mucus</td>
<td></td>
</tr>
</tbody>
</table>

⚠️ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

⚠️ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product’s page at [thermofisher.com](http://thermofisher.com). Wear appropriate protective eyewear, clothing, and gloves.

**Information about the product**

**Description of the product**

The Applied Biosystems™ VetMAX™ C. burnetii & Chlamydyphila spp. Kit is a molecular diagnostic tool enabling simultaneous real-time PCR detection of *Coxiella burnetii* and *Chlamydophila* spp.

Each DNA sample obtained after extraction is analyzed in a single well: the same well is used for specific detection of the bacterial DNA of *Coxiella burnetii* and *Chlamydophila* spp. and for the detection of an IPC (Internal Positive Control). A positive IPC reflects both the efficiency of extraction and the absence of inhibitor in the samples. It can be used on bacterial DNA extracted from placenta; placental, vaginal, or cervical swabs; amniotic fluid; milk; and vaginal mucus. Complete protocols for bacterial DNA extraction from these matrices are available on request from Technical Support.

**Kit contents and storage**

The VetMAX™ C. burnetii & Chlamydyphila spp. Kit contains reagents for triplex detection of *Coxiella burnetii*, *Chlamydophila* spp., and an internal control IPC. Upon receipt, the whole kit should be stored between −30°C and −10°C. After initial use of a component, store it according to the following recommendations:

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Volume (100 reactions)</th>
<th>Storage Upon receipt</th>
<th>Storage After initial use</th>
</tr>
</thead>
</table>
| 1 - Sequences Cox.b./Chlam (Green tube) | Sequence pool (primers and probes). Contains:  
- The detection system for the *Coxiella burnetii* target, including a TaqMan™ probe labeled VIC™ – NFQ (Non-Fluorescent Quencher)  
- The detection system for the *Chlamydophila* spp. target, including a TaqMan™ probe labeled FAM™ – NFQ (Non-Fluorescent Quencher)  
- The detection system for the IPC, including a TaqMan™ probe labeled Cyanine Red – NFQ (Non-Fluorescent Quencher) | 2 × 150 µL | −30°C to −10°C | −30°C to −10°C |
| 2 - Master Mix Cox.b./Chlam (White tube) | Mix for TaqMan™ real-time PCR. Contains the buffer and the real-time PCR enzyme. | 2 × 625 µL | −30°C to −10°C | 2°C to 8°C |
| 4a - EPC Cox.b./Chlam (Brown tube) | External Positive Control: *Coxiella burnetii* and *Chlamydyphila* spp. positive control. | 2 × 90 µL | −30°C to −10°C | −30°C to −10°C |

**Extraction and amplification controls**

The VetMAX™ C. burnetii & Chlamydyphila spp. Kit contains 1 control enabling validation of the amplification of the bacterial DNA:

4a - EPC Cox.b./Chlam: *Coxiella burnetii* and *Chlamydyphila* spp. positive control

A positive result within the specified C<sub>r</sub> range validates the amplification of the *Coxiella burnetii* and *Chlamydyphila* spp. target by real-time PCR.

Validation of nucleic acid extraction for each sample is done by detection of an endogenous IPC (Internal Positive Control), present in each sample.

A positive IPC result with a compliant value in a sample validates the extraction of this sample, whether positive or negative for the target pathogen, thus eliminating false negatives and verifying the inhibitor effect.

We recommend including two negative controls to confirm correct analysis:

**NCS: negative extraction control**

This control consists of components used in the extraction without addition of the sample (the sample volume can be replaced by the buffer used in the sample preparation or by DNase/RNase-free water), which undergoes the same treatment as the samples, namely nucleic acid extraction then real-time PCR.

A negative result for *Coxiella burnetii*, *Chlamydyphila* spp. and the endogenous IPC confirms proper lysis progression and absence of contamination during both extraction and real-time PCR.

**For Veterinary Use Only. For In Vitro Use Only.**
NC: negative amplification control
This is the amplification mix deposited on the plate during the preparation of the real-time PCR, with 5 µL of DNase/RNase-free water added to adjust the reaction to 25 µL.
A negative result for Coxiella burnetii, Chlamydophila spp., and the IPC confirms the absence of contamination during real-time PCR reaction preparation.

Materials required but not provided
Unless otherwise indicated, all materials are available through thermofisher.com.
• Precision micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
• DNase/RNase-free water
• 1X TE and 1X PBS buffers
• A real-time PCR thermal cycler capable of detecting the following fluorophores:
  - FAM™ (maximum emission: λ515 nm)
  - VIC™ (maximum emission: λ554 nm)
  - Cyanine Red (maximum emission: λ670 nm)
• Optical-quality consumables compatible with the thermal cycler used: PCR 96-well plates, PCR strips (8 or 12 wells), microtubes or capillaries; suitable plate covers or caps for capping

Analysis procedure
The real-time PCR reaction volume is 25 µL:
• Mix TFQQCHP: 20 µL per reaction. To be reconstituted extemporaneously before real-time PCR.
• Extracted DNA: 5 µL per analysis.

Extraction of bacterial DNA
DNA must be isolated from the samples for real-time PCR analysis.
NOTE: To learn about compatible and validated extraction methods for the VetMAX™ C. burnetii & Chlamyphila spp. Kit, please contact Technical Support.

Reconstitution of the reaction mix
Reconstitute the Mix TFQQCHP just before use in a room dedicated to preparation of the mix:
1. On first use, thaw the tube of 2 - Master Mix Cox b./Chlam at 2°C to 8°C on ice or on a refrigerated rack. Store and maintain between 2°C and 8°C for further use.
2. Thaw the tube of 1 - Sequence Cox b./Chlam at room temperature. Return it to between −30°C and −10°C after use.
3. Reconstitute the reaction mix Mix TFQQCHP at 2°C to 8°C on ice or on a refrigerated rack according to the following calculation table:

<table>
<thead>
<tr>
<th>Component</th>
<th>For 1 reaction</th>
<th>For N reactions[1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Sequence Cox b./Chlam</td>
<td>3 µL</td>
<td>N × 3 µL</td>
</tr>
<tr>
<td>2 - Master Mix Cox b./Chlam</td>
<td>12.5 µL</td>
<td>N × 12.5 µL</td>
</tr>
<tr>
<td>DNase/RNase-free water</td>
<td>4.5 µL</td>
<td>N × 4.5 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>20 µL</td>
<td>N × 20 µL</td>
</tr>
</tbody>
</table>

[1] It is recommended to allow for an additional reaction with respect to the total number of reactions to be carried out during the analysis [samples and controls]. Never mix components from different lots of kits (see Certificate of Analysis).
4. After reconstitution, start the real-time PCR immediately. Keep the Mix TFQQCHP at 2°C to 8°C on ice or on a refrigerated rack until use.

Preparation of the real-time PCR
1. Create an analysis plan for distribution of the mixes and samples. Keep the positive control (EPC) away from the other samples if possible.
2. Mix the Mix TFQQCHP by gentle agitation, then centrifuge briefly.
3. Add 20 µL of Mix TFQQCHP to each PCR plate well, PCR strip or capillary used.
4. Add the DNA from samples and controls to the reaction mix, according to the following preset analysis plan:

<table>
<thead>
<tr>
<th>Type of analysis</th>
<th>Component</th>
<th>Sample volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample for analysis</td>
<td>DNA extracted from the sample</td>
<td>5 µL</td>
</tr>
<tr>
<td>Positive amplification control</td>
<td>4a - EPC Cox b./Chlam</td>
<td>5 µL</td>
</tr>
<tr>
<td>Negative extraction control (NCS)</td>
<td>Extracted NCS</td>
<td>5 µL</td>
</tr>
<tr>
<td>Negative amplification control (NC)</td>
<td>DNase/RNase-free water</td>
<td>5 µL</td>
</tr>
</tbody>
</table>
5. Cover the PCR plate, PCR strips or capillaries with an adhesive plate cover or suitable caps.

Amplification by real-time PCR
1. Create the following detectors on the thermal cycler:

<table>
<thead>
<tr>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>COXB</td>
<td>VIC™</td>
</tr>
<tr>
<td>CHLAM</td>
<td>FAM™</td>
</tr>
<tr>
<td>IPC</td>
<td>Cyanine Red</td>
</tr>
<tr>
<td>Passive reference: ROX™</td>
<td></td>
</tr>
</tbody>
</table>

[1] The fluorophore ROX™ is required for real-time PCR analysis if the thermal cycler is capable of detecting it. For other thermal cyclers, absence of the ability to detect this fluorophore does not affect the analysis by real-time PCR.
2. Assign the COXB, CHLAM, and IPC detector to each sample well used in the analysis.
3. Set up the following real-time PCR program for the analysis:
Step repetitions | Temperature | Duration
--- | --- | ---
Step 1 | ×1 | 50°C | 2 minutes
Step 2 | ×1 | 95°C | 10 minutes
Step 3 | ×45 | 95°C | 15 seconds
| | | 60°C | 1 minute

(1) Collection of fluorescence data during the 60°C – 1 minute stage.

4. Place the PCR plate, the PCR strips or the capillaries in the thermal cycler and run the real-time PCR.

**Analysis of the results**

**Analysis of the raw data**

Refer to the recommendations of the thermal cycler manufacturer for the analysis of the raw data.

1. Position the threshold limits separately for each target of the real-time PCR.
2. For each detector, interpret the results according to the sample Ct values obtained as recommended below.

**Validation**

The run is validated if the following criteria are met:

<table>
<thead>
<tr>
<th>COXB detector</th>
<th>CHLAM detector</th>
<th>IPC detector</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC COXCHL</td>
<td>C&lt;\textsubscript{T} = \text{C}\textsubscript{T} \pm 3C&lt;\textsubscript{t}(1)</td>
<td>C&lt;\textsubscript{T} = \text{C}\textsubscript{T} \pm 3C&lt;\textsubscript{t}(1)</td>
<td>C&lt;\textsubscript{T} &lt; 45 or C&lt;\textsubscript{T} &gt; 45(2)</td>
</tr>
<tr>
<td>NCS</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
</tr>
<tr>
<td>NC</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
</tr>
</tbody>
</table>

(1) Refer to the values listed in section “EPC” of the Certificate of Analysis for the lot used for the test.

(2) The IPC value in the EPC should not be used for test validation.

**Interpretation of results**

For each sample analyzed, the results should be interpreted as shown below:

<table>
<thead>
<tr>
<th>COXB detector</th>
<th>CHLAM detector</th>
<th>IPC detector</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;\textsubscript{T} &lt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45 or C&lt;\textsubscript{T} &gt; 45</td>
<td>Coxiella burnetii and Chlamydophila positive</td>
</tr>
<tr>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45 or C&lt;\textsubscript{T} &gt; 45</td>
<td>Coxiella burnetii positive</td>
</tr>
<tr>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45 or C&lt;\textsubscript{T} &gt; 45</td>
<td>Chlamydophila positive</td>
</tr>
<tr>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45 or C&lt;\textsubscript{T} &gt; 45</td>
<td>Negative</td>
</tr>
<tr>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &gt; 45</td>
<td>C&lt;\textsubscript{T} &lt; 45</td>
<td>Not validated(1)</td>
</tr>
</tbody>
</table>

(1) The sample will be returned as not validated due to the negative IPC.

**Procedure for handling non-validated samples**

1. Dilute the non-validated sample DNA at a 1:10 dilution in 1X TE buffer.
2. Perform a new PCR analysis on 5 µL of this dilution.
3. If the diluted DNA is positive for *Coxiella burnetii* or *Chlamydia phila* or negative for *Coxiella burnetii* and *Chlamydia phila* with a compliant IPC result, the obtained result is then validated.
4. If the diluted DNA is negative for *Coxiella burnetii* and *Chlamydia phila* with a non-compliant IPC result, the obtained result is still not validated. In this case, repeat the nucleic acid extraction using the sample pre-diluted 1:10 in 1X PBS buffer before extraction.
5. If the result is still not validated, repeat the analysis on a new sample.
Documentation and support

Customer and technical support
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- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision history of Pub. No. MAN0008883 (English)

<table>
<thead>
<tr>
<th>Rev.</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
</table>
| E.0  | 03 November 2021 | • The validation criteria for the IPC detector in the EPC was updated.  
• References to the qualitative application were removed.  |
| D.0  | 9 August 2021 | • Removed instructions for the quantitative application.  
• Updated the External Positive Control (4a – EPC Cox b./Chlam). |
| C.0  | 30 May 2017 | Updated to the current document template, with associated updates to the warranty, trademarks, and logos. |
| B.0  | 16 October 2014 | Correction of the quenchers and reagents volumes |
| A.0  | April 2014 | New document |

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