PrioCHECK™ Ruminant Q Fever Ab Plate Kit

Immunoenzymatic test for specific detection of anti-\textit{Coxiella burnetii} antibodies in ruminant serum and milk

**Catalog Numbers** ELISACOXLS2, ELISACOXLS5

**Doc. Part No.** 100020257  **Pub. No.** MAN0007464  **Rev.** B.0

<table>
<thead>
<tr>
<th>Technology</th>
<th>Species</th>
<th>Sample matrix</th>
<th>Sample type</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-well indirect ELISA</td>
<td>Bovine</td>
<td>Serum</td>
<td>Individual</td>
<td>Short incubation</td>
</tr>
<tr>
<td></td>
<td>Ovine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caprine</td>
<td>Milk</td>
<td>Individual Tank</td>
<td>Long incubation</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 \url{thermofisher.com/support}.

**WARNING!** POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product’s page at \url{thermofisher.com}. Wear appropriate protective eyewear, clothing, and gloves.

**General information**

The antigen (antigen phase I + II) in the Applied Biosystems™ PrioCHECK™ Ruminant Q Fever Ab Plate Kit was isolated by the French National Institute for Agricultural Research (INRA) in Nouzilly from domestic ruminants. This ovine strain of \textit{Coxiella burnetii} is responsible for abortion in ovines. Validations performed by INRA and in-house show that the PrioCHECK™ Ruminant Q Fever Ab Plate Kit exhibits greater sensitivity for detecting \textit{Coxiella burnetii} shedding animals than the Nine Mile ELISA kits.

**Procedure overview**

The test is based on the principle of an **indirect ELISA assay**.

1. Samples and controls are distributed in the plate coated with the \textit{Coxiella burnetii} antigen. Any specific anti-\textit{Coxiella burnetii} antibodies bind to the antigen.
2. After washing, a G protein conjugate labeled with peroxidase (HRP) is added, binding to the antibodies previously attached to the plate.
3. The unbound conjugate is eliminated by washing, followed by addition of a chromogenic substrate. A blue color results from substrate oxidation by the HRP-conjugate.
4. After stopping the reaction, the color turns yellow. The results are read by an ELISA plate reader. The intensity of the yellow color present in the positive samples is proportional to the amount of specific antibodies in the sample.
Kit contents and storage

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>ELISACOXLS2 (192 tests)</th>
<th>ELISACOXLS5 (480 tests)</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Coated microplate Q Fever</td>
<td>Q Fever-coated microplate, 12 strips of 8 wells</td>
<td>2 units</td>
<td>5 units</td>
<td>5±3°C(1)</td>
</tr>
<tr>
<td>2a - Serum Negative C. Q Fever</td>
<td>Q Fever negative control (NC) serum</td>
<td>250 µL</td>
<td>600 µL</td>
<td>5±3°C</td>
</tr>
<tr>
<td>2b - Milk Negative C. Q Fever</td>
<td>Q Fever negative control (NC) milk</td>
<td>5 mL</td>
<td>12 mL</td>
<td></td>
</tr>
<tr>
<td>3 - Positive C. Q Fever</td>
<td>Q Fever positive control (PC) serum</td>
<td>250 µL</td>
<td>600 µL</td>
<td>5±3°C</td>
</tr>
<tr>
<td>4 - Conjugate (100x) Q Fever</td>
<td>G protein-HRP Conjugate Q Fever (red), 100X concentrate</td>
<td>500 µL</td>
<td>900 µL</td>
<td>5±3°C(2)</td>
</tr>
<tr>
<td>A - Wash (10x)</td>
<td>Wash solution, 10X concentrate</td>
<td>125 mL</td>
<td>250 mL</td>
<td>5±3°C</td>
</tr>
<tr>
<td>B1 - Sample DB Q Fever</td>
<td>Q Fever sample dilution buffer (green)</td>
<td>120 mL</td>
<td>250 mL</td>
<td></td>
</tr>
<tr>
<td>B2 - Conjugate DB Q Fever</td>
<td>Q Fever conjugate dilution buffer</td>
<td>50 mL</td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>C - Substrate</td>
<td>Substrate solution</td>
<td>24 mL</td>
<td>60 mL</td>
<td></td>
</tr>
<tr>
<td>D - Stop</td>
<td>Stop solution</td>
<td>24 mL</td>
<td>60 mL</td>
<td></td>
</tr>
<tr>
<td>Adhesive plate covers</td>
<td></td>
<td></td>
<td></td>
<td>RT(3)</td>
</tr>
</tbody>
</table>

(1) Unused strips can be stored in the sealed pouch with desiccant (supplied with the kit) at 5±3°C until the kit’s expiration date.
(2) The diluted conjugate solutions should be used immediately after preparation.
(3) Room temperature

Materials required but not provided

Unless otherwise indicated, all materials are available through thermofisher.com.

<table>
<thead>
<tr>
<th>Single and multi-channel micropipettes</th>
<th>Distilled or deionized water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dilution plates</td>
<td>Disposable containers</td>
</tr>
<tr>
<td>Microplate incubator (37±2°C)</td>
<td>ELISA reader equipped with a 450 nm filter or 620 nm filters</td>
</tr>
<tr>
<td>Disposable pipette tips</td>
<td>450 and 620 nm filters</td>
</tr>
</tbody>
</table>

Important procedural guidelines

- Do not mix reagents from different kit batches.
- Avoid contaminating the reagents by using single-use sampling equipment.
- Do not pipette reagents by mouth.

Preparation of samples

**Serum:** Fresh, refrigerated serum (8 days at 5±3°C) or frozen serum (1 year at < −16°C) can be used. The pre-homogenized samples and controls are tested at a 1:400 dilution.

**Milk:** Fresh, refrigerated milk (individual or tank) (8 days at 5±3°C) or frozen milk (1 year at < −16°C) can be used with or without an added preservative. The pre-homogenized samples and controls are tested at a 1:20 dilution.

**NOTE:** The use of an internal tracer is highly recommended for each test series. An internal reference (Cat. No. RFQS) is available for serum or milk use.

Preparation of reagents

- Reagents 1 - Coated microplate Q Fever, B1 - Sample DB Q Fever, B2 - Conjugate DB Q Fever, C - Substrate and D - Stop are ready for use.
- Reagents 2a - Serum Negative C. Q Fever, 2b - Milk Negative C. Q Fever and 3 - Positive C. Q Fever (for serum use only) are tested as samples.

**Milk Positive Control Q Fever** is prepared from a 1:20 dilution of reagent 3 - Positive C. Q Fever. Example: 10 µL of reagent 3 - Positive C. Q Fever + 190 µL of reagent 2b - Milk Negative C. Q Fever. Mix after diluting. The Milk Positive Control Q Fever solution should be used immediately after dilution and tested as a sample.

- A - Wash (10x) solution should be diluted to 1:10 in distilled/deionized water.
  **Example:** for one strip: 2 mL of A - Wash (10x) solution in 18 mL of water; for one plate: 25 mL of A - Wash (10x) solution in 225 mL of water. Mix after diluting. The diluted Wash solution can be stored for 1 month at 5±3°C.
  **NOTE:** Due to the high salt concentration, crystals may form in the A - Wash (10x) solution. Prior to dilution, shake the bottle to dissolve any crystals.

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2 PrioCHECK™ Ruminant Q Fever Ab Plate Kit Instructions for Use
- Reagent 4 - Conjugate (100x) Q Fever should be diluted to 1:100 in B2 - Conjugate DB Q Fever reagent. Mix after diluting. Use the diluted Conjugate Q Fever solution immediately after dilution.

**Perform the ELISA test**

**NOTE:** Bring the reagents to room temperature (21±4°C) before performing the test. The tolerance range for incubation times is ±10%. The use of disposable containers is recommended for distribution of components.

### 1. Distribution of controls and samples

**Serum samples and controls are tested diluted to 1:400 in B1 - Sample DB Q Fever reagent:**

A. In a pre-dilution plate, pre-dilute the serum samples and controls to 1:20 in B1 - Sample DB Q Fever reagent:
   - Add 5 µL of each serum to the wells of the pre-dilution plate. Keep the same order as the one that will be used on the coated plate.
   - Add 95 µL of reagent B1 - Sample DB Q Fever to the wells containing the controls and samples. Gently shake the plate.
   - Incubate at room temperature for 5 minutes before transferring to the coated plate.

**NOTE:** The pre-diluted serums can be stored for 24 hours at 5±3°C.

B. To obtain the final 1:400 dilution, perform a 2nd dilution to 1:20 in B1 - Sample DB Q Fever reagent in the coated plate:
   - Add 5 µL of reagent 2a - Serum Negative C. Q Fever pre-diluted to 1:20 to wells A1 and B1 (for example).
   - Add 5 µL of reagent 3 - Positive C. Q Fever pre-diluted to 1:20 to wells C1 and D1 (for example).
   - Add 5 µL of serum sample pre-diluted to 1:20 to the remaining wells.
   - Add 95 µL of reagent B1 - Sample DB Q Fever to each well containing the controls or samples. Gently shake, then cover the plate with an adhesive plate cover. **Incubate the plate for 1 hour at 37±2°C.**

**Milk samples and controls are tested diluted to 1:20 in B1 - Sample DB Q Fever reagent:**

- Add 5 µL of reagent 2b - Milk Negative C. Q Fever to wells A1 and B1 (for example).
- Add 5 µL of Milk Positive Control Q Fever solution (see “Preparation of reagents”) to wells C1 and D1 (for example).
- Add 5 µL of milk to the remaining wells.
- Add 95 µL of reagent B1 - Sample DB Q Fever to each well containing the controls or samples. Gently shake, then cover the plate with an adhesive plate cover. **Incubate the plate overnight (16 to 18h) at 5±3°C.**

### 2. Washing steps (3 washes)

Empty the plate and perform 3 washes with the diluted Wash solution (see “Preparation of reagents”) using 300 µL per well. Empty and tap the plate on absorbent paper to eliminate any traces of liquid. Washes can be performed either manually or automated using a plate washer. Do not allow the plate to dry out.

### 3. Distribution of conjugate

Add 100 µL of diluted Conjugate Q Fever solution (see “Preparation of reagents”) to each well. Gently shake the plate, and cover the plate using a new adhesive plate cover. **Incubate the plate for 1 hour at 37±2°C.**

### 4. Washing steps (3 washes)

Repeat the Washing steps (step 2) as described above.

### 5. Test development

Add 100 µL of solution C - Substrate to each well. Gently shake the plate for 2 seconds. **Incubate for 10 minutes at room temperature (21±4°C) in darkness.** Do not cover the plate.
Add 100 µL of solution D - Stop to each well and in the same order as solution C - Substrate. Gently shake the plate for 2 seconds.

### 6. Reading

Dry the bottom of the plates with a soft tissue to remove any dust. Read the plate within 30 minutes after stopping the reaction at 450 nm (monochromatic) or at dual wavelengths of 450–620 nm on a microplate reader.
Calculation
Calculate the average OD (Optical Density) of the PC (OD_{m PC}) and that of the NC (OD_{m NC}).
For each sample, calculate the S/P (Sample/Positive) ratio:
\[
S/P = \frac{(OD_{Sample} - OD_{m NC})}{(OD_{m PC} - OD_{m NC})}
\]
\[
Titer = S/P \times 100
\]
NOTE: For negative samples, S/P ratios may be < 0.

Validation
The test is validated if:
\[
OD_{m PC} > 0.400 \quad \text{and} \quad OD_{m PC}/OD_{m NC} > 2
\]

Interpretation of results

<table>
<thead>
<tr>
<th>Table 1 Serum</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer ≤ 40</td>
<td>Negative</td>
</tr>
<tr>
<td>40 &lt; Titer ≤ 100</td>
<td>Positive +</td>
</tr>
<tr>
<td>100 &lt; Titer ≤ 200</td>
<td>Positive ++</td>
</tr>
<tr>
<td>200 &lt; Titer ≤ 300</td>
<td>Positive +++</td>
</tr>
<tr>
<td>Titer &gt; 300</td>
<td>Positive ++++</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Individual milk</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer ≤ 40</td>
<td>Negative</td>
</tr>
<tr>
<td>40 &lt; Titer ≤ 100</td>
<td>Positive +</td>
</tr>
<tr>
<td>100 &lt; Titer ≤ 200</td>
<td>Positive ++</td>
</tr>
<tr>
<td>Titer &gt; 200</td>
<td>Positive ++++</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3 Tank milk</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer ≤ 30</td>
<td>Negative</td>
</tr>
<tr>
<td>30 &lt; Titer ≤ 100</td>
<td>Positive +</td>
</tr>
<tr>
<td>100 &lt; Titer ≤ 200</td>
<td>Positive ++</td>
</tr>
<tr>
<td>Titer &gt; 200</td>
<td>Positive ++++</td>
</tr>
</tbody>
</table>

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