PrioCHECK™ Ruminant Q Fever Ab Plate Kit

Immunoenzymatic test for specific detection of anti-*Coxiella burnetii* antibodies in ruminant serum and milk

Catalog Numbers ELISACOXLS2, ELISACOXLS5
Doc. Part No. 100020257 **Pub. No.** MAN0007464 **Rev.** B.0

Technology	Species	Sample matrix	Sample type	Protocol
Single-well indirect ELISA - Strip plates	Bovine Ovine Caprine	Serum	Individual	Short incubation
		Milk	Individual Tank	Long incubation

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.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **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 *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 *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 *Coxiella burnetii* antigen. Any specific anti-*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

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

[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**.

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

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.

- 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:

$$S/P = (OD_{Sample} - OD_{m\ NC}) / (OD_{m\ PC} - OD_{m\ NC})$$

$$Titer = S/P \times 100$$

NOTE: For negative samples, **S/P** ratios may be **< 0**.

Validation

The test is validated if:

$OD_{m\ PC} > 0.400$ and $OD_{m\ PC} / OD_{m\ NC} > 2$

Interpretation of results

Table 1 Serum

Results	Interpretation
Titer ⩽ 40	Negative
40 < Titer ⩽ 100	Positive +
100 < Titer ⩽ 200	Positive ++
200 < Titer ⩽ 300	Positive +++
Titer > 300	Positive ++++

Table 2 Individual milk

Results	Interpretation
Titer ⩽ 40	Negative
40 < Titer ⩽ 100	Positive +
100 < Titer ⩽ 200	Positive ++
Titer > 200	Positive +++

Table 3 Tank milk

Results	Interpretation
Titer ⩽ 30	Negative
30 < Titer ⩽ 100	Positive +
100 < Titer ⩽ 200	Positive ++
Titer > 200	Positive +++

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- 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.

Limited product warranty

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Rev.	Date	Description
B.0	15 June 2016	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	January 2014	• Corrected number of washes in steps 2 and 4. • Changed dilution ratio from fraction style to colon style. • Added revision history table.
1.0	May 2013	Life Technologies format document.