MONOSCREEN Ab ELISA

Instructions for use
BIOK298-Coxiella_NO_(EN)_V04
29/06/2026

Monoscreen AbELISA *Coxiella burnetii*

Reference: BIO K 298

ELISA Kit for the serodiagnosis of Q fever

Monowell, indirect test

In vitro and strictly veterinary use

Sample / dilution	Bovine	Caprine	Ovine
Blood sera / 100X	✓	✓	✓
Milk* / 1X	✓	✗	✗

* Preparation : centrifugation at 4000 g for 20 minutes, take up the middle layer of liquid.

Presentation

Product reference	BIO K 298/2
Format	2 plates, strip of 8 wells
Reactions	192 tests

Composition of the kit

Provided material		Code	Type*	BIO K 298/2
Microplate	Microplate	D00876	1	2
Washing solution (20X)	Washing solution (20X)	D00695	A	1 X 100 mL
Dilution solution (1X)	Colored dilution solution (1X)	D01511	A	3 X 125 mL
TMB solution (1X)	Single component TMB (1X)	D01585	A	1 X 30 mL
Stop solution (1X)	Stop solution (1X)	D00680	A	1 X 30 mL
Conjugate (50X)	Conjugate (50X)	D01596	1	1 X 0,6 mL
CTL POS	Positive control	D01044	a	1 X 0,5 mL
CTL NEG	Negative control	D01030	a	1 X 0,5 mL

*: (1): dependent on kit and batch : (a): dependent on kit / (A): substitutable with components A / (B): substitutable with components B.

Revision history

Date	Version	Modifications
01/06/2022	V03	Formatting and simplification of the entire instructions for use
29/06/2026	V04	Adaptation of component volume. Distribution of stop solution modified from 50 µL to 100 µL.

Note: minor changes to typography, grammar and formatting are not included in the revision history.

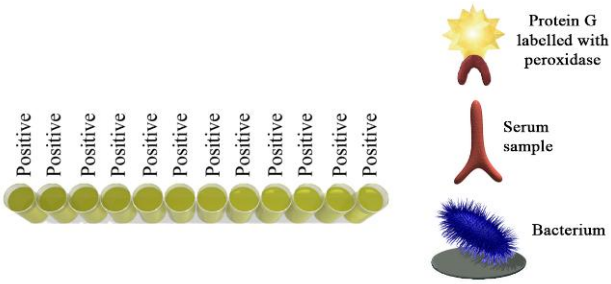
A. Introduction

Q fever mainly affects humans, cattle, sheep and goats. The etiological agent, *Coxiella burnetii* is a Gram-negative intracellular bacterium which multiplies in the macrophage phagolysosomes. *Coxiella burnetii* can occur in two antigenic forms: a pathogenic phase I, isolated from infected animals or individuals, and an avirulent phase II, obtained *in ovo* or *in vitro*. There are 2 forms of infection, acute and chronic, which have different serological profiles: during the acute phase of the disease, titers of type IgG antibodies are high against phase II, while during the chronic phase of the disease, elevated levels of anti-phase I and II IgG antibodies are observed. In cows, sheep and goats, Q fever has mostly been associated with late abortions and reproductive disorders such as premature birth, dead or weakened fetuses, metritis, and infertility. Nevertheless, in a given species the serological responses or the isolation of the bacterium do not necessarily correlate with the expression of the clinical disease. Serological analyzes are appropriate for screening herds, but the interpretation at the individual level can be difficult.

B. Test principle

The entire 96-well microplates were sensitized with antigenic extracts of *Coxiella burnetii* in phase I + II. Serums, plasmas and controls are diluted in the dilution solution. The milks are used pure. After 60 minutes of incubation and a washing step, the protein G conjugated to peroxidase is added. After 60 minutes of incubation and a washing step, the operator adds the chromogen tetramethylbenzidine (TMB).

In case of presence of specific immunoglobulins of *Coxiella burnetii* in the serum, plasma or milk, the conjugate (antibody-antigen complex) remains attached to the well containing the bacterial antigen and the enzyme catalyzes the transformation of the colorless chromogen into a blue product. The intensity of the color is proportional to the content of specific antibodies present in the sample.



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20µL, 20-200µL et 100-1000µL range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer
- Incubator at 21±3°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …

Additional kit

- **Tracer *Coxiella burnetii* (Ref. : BDE K 298).** Internal reference material for Q fever serology by ELISA.

D. Precautions for use

- The reagents must be kept between +2 et +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1 M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution solution is colored in yellow. It is used for dilution of samples, positive and negative controls, and conjugate.
- The conjugate must be diluted 50-fold in the dilution solution.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- The **blood sera** samples and kit **controls** (the positive, and negative controls) must be diluted **100-fold** in the dilution solution and homogenized.
  Avoid using haemolysed or coagulated samples.

  Recommended dilution:
  10 µL of sample + 990 µL of dilution solution.

- **Milk** samples are used pure, i.e., **undiluted**.

G. Procedure

- Bring all the reagents to **21±3°C** before use.
- Carefully read through the previous points.

***N.B.** : To avoid differences in incubation time between samples, it is possible to prepare sample and reference dilutions or to distribute milk samples in a dilution microplate before transfer (100 µL) into the test microplate using a multi-channel pipette.*

Serum protocol (1/100 dilution)

1. Distribute **100 µL per well** of **diluted serum samples** and **kit controls.** Cover and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.

Milk protocol (no dilution)

2. Distribute **100 µL per well** of **pure milk** samples and **diluted** kit **controls.** Cover and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.

Joint protocol

3. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL of washing solution per well**. Avoid the formation of bubbles in the wells and the desiccation of the microplate between each wash.

4. Add **100 µL of diluted conjugate** per well. Cover and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.

5. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL of washing solution per well**. Avoid the formation of bubbles in the wells and the desiccation of the microplate between each wash.

6. Distribute **100 µL** of **TMB solution** per well. Incubate at **21 ± 3°C** during **10 ± 1 min** away from the light, without covering.

7. Distribute the **stopping solution** at rate of **100 µL per well**. Color changes from blue to yellow.

8. Record the optical densities using a plate spectrophotometer with a **450 nm filter** within 5 minutes after adding the stopping solution.

H. Validation of results

The test can only be **validated** if:

- The difference between positive and negative control optical density (OD) readings is greater than 1,000.

$$OD_{\text{positive control}} - OD_{\text{negative control}} > 1{,}000$$

- The negative control optical density reading is less than 0,400.

$$OD_{\text{negative control}} < 0{,}400$$

I. Interpretation of results

Calculate for each sample its coefficient (S/P %) using the following formula :

$$S/P\ \% = \frac{OD_{\text{sample}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}} * 100$$

	Results	Status
Bovine, caprine, and ovine serum	S/P % < 40%	Negative
	40% ≤ S/P % ≤ 60%	Doubtful
	S/P % > 60%	Positive
Bovine milk	S/P % < 30%	Negative
	30% ≤ S/P % ≤ 60%	Doubtful
	S/P % > 60%	Positive

Get the interpretation of your results quickly and easily using **AnalysiScreen**, our free online platform, available on our website : https://www.biox.com.

ANALYSISCREEN

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysiScreen™** is :

- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

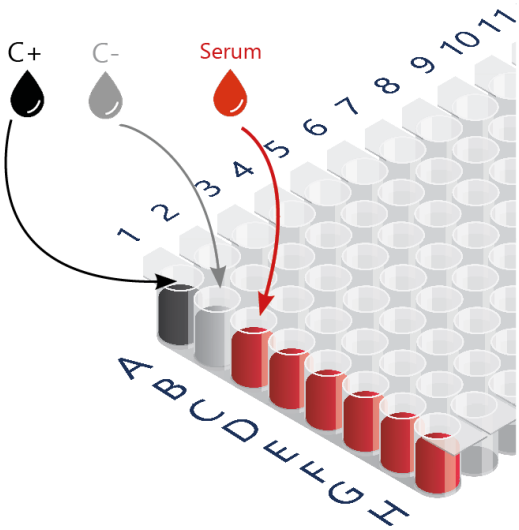
Symbols

Symbol	Meaning
REF	Catalog number
	Manufacturer
	Temperature limit
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	Keep away from light
	Keep dry
	Corrosive substance
	Hazardous/irritating product

Notes*

Serum protocol

1 Distribute 100 µL of diluted samples 1/100
Distribute 100 µL of the diluted kit controls (positive and negative) 1/100



Kit microplate

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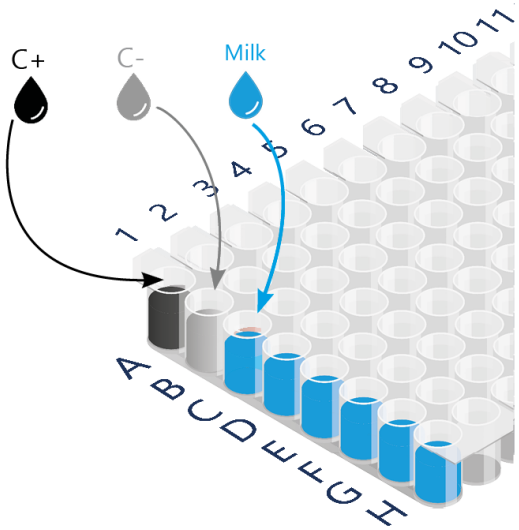
1 Distribute 100 µL of centrifuged non diluted samples
Distribute 100 µL of diluted kit controls (positive and negative) 1/100



Kit microplate

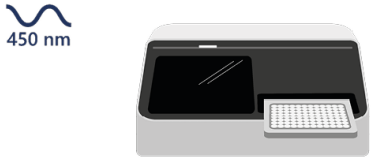
Joint protocol

2 Add 100 µL of diluted conjugate (1/50)

3 Add 100 µL of TMB

4 Add 100 µL of stopping solution

5 Record the optical densities



* Notes do not replace the instructions for use of which they are a synthesis.

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