MONOSCREEN Ab ELISA

Monoscreen AbELISA Coxiella blocking phase I

Reference : BIO K 001

ELISA Kit for serodiagnosis of Q fever
Monowell, blocking test

In vitro and strictly veterinary use

Sample / dilution	Cattle	Caprin	Ovin
Serum / 2X	✓	✓	✓
Milk / 2X	✓	✓	✗

Presentation

Product reference	BIO K 001/2
Format	2 plates, strip of 8 wells
Reactions	192 tests

Composition of the kit

Provided material		BIO K 001/2
Microplate	Microplates	2
Washing solution	Washing solution (20X)	1 X 100 mL
Dilution solution	Colored dilution solution (1X)	1 X 100 mL
Conjugate	Conjugate (1X)	1 X 25 mL
CTL POS	Positive control	1 X 0,5 mL
CTL NEG	Negative control	1 X 0,5 mL
TMB solution	Single component TMB (1X)	1 X 25 mL
Stop solution	Stop solution (1X)	1 X 15 mL

Revision history

Date	Version	Modifications
13/04/2023	V01	First version
23/05/2023	V02	Addition of caprine milk, monoclonal antibody specificity

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 that multiplies in the phagolysosomes of macrophages.

Coxiella burnetii can present 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*.

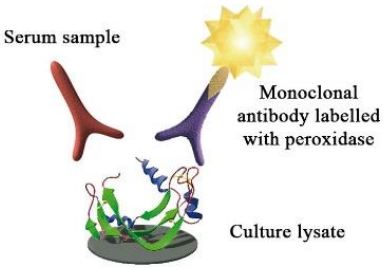
There are 2 forms of infection, acute and chronic, which have different serological profiles: during the acute phase of the disease, IgG antibody titers are high against phase II, while during the chronic phase of the disease, high levels of IgG antibodies against phases I and II are observed.

In cows, sheep and goats, Q fever has been associated mainly with late abortions and reproductive disorders such as premature births, dead or weakened fetuses, metritis, and infertility. However, in a given species serological responses or isolation of the bacterium do not necessarily correlate with the expression of clinical disease. Serological tests are appropriate for herd screening, but interpretation at the individual level can be difficult.

B. Test principle

The test uses 96-well microtitration plates sensitized by *Coxiella burnetii extract*. The operator deposits the previously diluted test sera in the microplate's wells. After 120 minutes' incubation and a washing step, the operator adds the conjugate, which is a virenose specific monoclonal antibody against phase 1 *Coxiella burnetii* coupled to peroxidase. After incubating and washing the preparation, the operator adds the chromogen tetramethylbenzidine (TMB).

This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the color is inversely proportionate to the sample's serum titer. Positive and negative control sera are provided with the kit to be able to validate the test results.



C. Material required but 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 37±2°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …

D. Warnings and precautions of 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.
- Do not use reagents from other kits.
- 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, kit controls, and conjugate.
- The conjugate is ready to use.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Serum samples** and kit controls (positive and negative control) are to be diluted **2 times** in the dilution solution, and homogenized.
  Avoid using hemolyzed or coagulated samples.

  Recommended dilution:
  50 µL of sample + 50 µL of dilution solution

- To skim the **milk**, samples are to be centrifuged **20 min at 4000g**. Take up the middle layer of liquid, taking care not to touch the underlying cell sediment.
  Milk samples are to be diluted **2 times** in the dilution solution.

G. Procedure

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

1. Distribute the **diluted serum and milk samples** and the **diluted kit controls** at a rate of **100 µL per well**.
   Cover and incubate the plate at **37 ± 2°C** during **120 ± 5 min**.

N.B.: To avoid differences in incubation time between samples, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (200 µL) into the test microplate using a multi-channel pipette.

2. 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.

3. Add **100 µL conjugate** per well. Cover with a lid and incubate the plate at **37 ± 2°C** during **30 ± 2 min**.

4. 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.

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

6. Distribute the **stopping solution** at rate of **50 µL per well**. Color changes from blue to yellow.

7. 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 readings is greater than 0,600.

$$OD_{\text{negative control}} - OD_{\text{positive control}} > 0{,}600$$

- the inhibition percentage of the positive control (%inh) is greater than 50%.

$$\%inh_{\text{positive control}} > 50\%$$

I. Interpretation of results

Calculate for each sample its inhibition percentage (%inh) using the following formula :

$$\%\ inh = \frac{OD_{\text{negative control}} - OD_{\text{sample}}}{OD_{\text{negative control}}} * 100$$

	Results	Status
Sample	%inh < 70%	Negative
	%inh ≥ 70 %	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

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Notes*

1 Distribute 50µL of dilution solution

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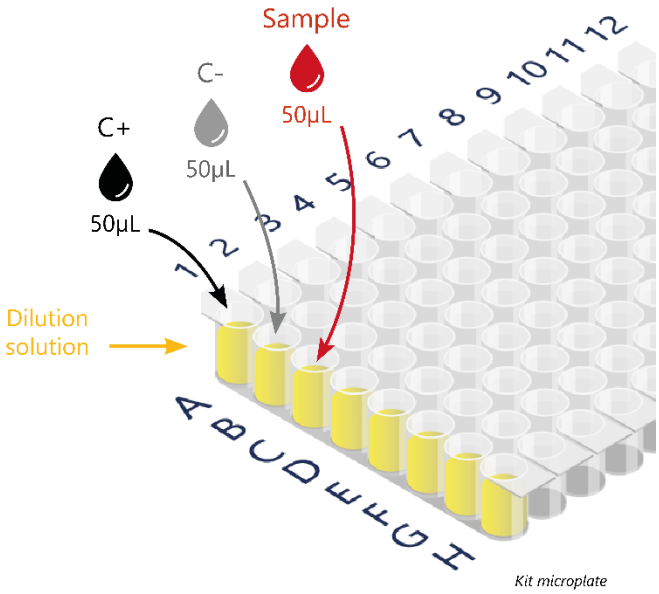
Add 50µL of serum samples and 50µL of positive and negative controls



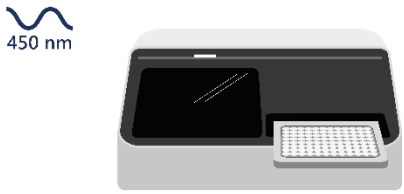
Kit microplate

2 Add 100 µL of conjugate

3 Add 100 µL of TMB solution

4 Add 50 µL of stopping solution

5 Record optical densities



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

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