MONOSCREEN Ab ELISA

Instruction manual
BIOK260-MYCB_NO_(EN)_V03
29/06/2026

Monoscreen AbELISA *Mycoplasma bovis*

Reference : BIO K 260

ELISA test for serodiagnosis of *Mycoplasma bovis*

Biwell, indirect test

For veterinary *in vitro* use only

Sample / dilution	Bovine
Serum/Plasma* / 100X	✓
Milk / 1X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 260/2	BIO K 260/5
Format	2 plates, strips of 16 wells	5 plates, strips of 16 wells
Reactions	96 tests	240 tests

Kit composition

Provided material		Type*	Code	BIO K 260/2	Code	BIO K 260/5
Microplate	Microplates	1	D00575	2	D00576	5
Washing solution (20X)	Washing solution (20X)	A	D00695	1 x 100 mL	D00696	1 x 250 mL
Dilution solution (1X)	Colored dilution solution (1X)	A	D01511	2 x 125 mL	D01511	2 x 250 mL
TMB solution (1X)	Single component TMB solution (1X)	A	D01585	1 x 30 mL	D01557	1 x 60 mL
Stop solution (1X)	Stopping solution (1X)	A	D00680	1 x 30 mL	D01556	1 x 60 mL
Conjugate (50X)	Conjugate (50X)	1	D01596	1 x 0,6 mL	D01563	1 x 1,5 mL
CTL POS	Positive control	a	D01033	1 x 0,5 mL	D01033	1 x 0,5 mL
CTL NEG	Negative control	a	D01030	1 x 0,5 mL	D01030	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
29/06/2026	V03	Layout and simplification of the entire manual. Adjustment of component volume. Distribution of stop solution modified from 50 µL to 100 µL.

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

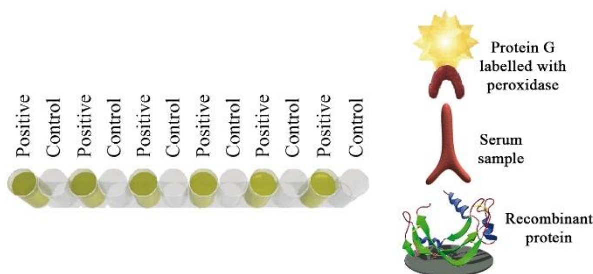
A. Introduction

Mycoplasma bovis is associated with many cattle diseases, including arthritis, pneumonia in calves and young stock, mastitis, and genital infections. The infectious pneumonias that affect intensively raised calves are responsible for sizable economic losses due to the mortality, treatment costs, and growth delays that they cause. These respiratory infections often involve multiple factors and are caused by interactions among viruses, mycoplasmas, and bacteria. Several species of *Mycoplasma* have been isolated from the respiratory tracts of calves. Some of them are most probably simple commensals or opportunistic species that merely worsen the lung damage caused by other agents. *Mycoplasma bovis* has been isolated from the lungs of calves with pneumonia. It is probably the most pathogenic species affecting the Bovidae after *Mycoplasma mycoides mycoides*. *Mycoplasma bovis* can induce the development of pneumonia in gnotobiotic calves. *Mycoplasma bovis* is frequently found in association with *Mannheimia haemolytica* in pneumonia in calves.

B. Test principle

The test uses 96-well microtitration plates sensitized by a recombinant protein from *Mycoplasma bovis* expressed by *E. coli*. A gene from *Mycoplasma bovis* is expressed by this recombinant *E.coli* culture. The plate's odd columns (1, 3, 5, 7, 9, 11) contain the recombinant protein, whereas the even columns (2, 4, 6, 8, 10, 12) contain a negative control antigen. We thus have a genuine negative control to differentiate the specific antibody. Using such a control reduces the number of false positives considerably.

The test blood sera and plasma are diluted in the dilution solution. The milk samples are used undiluted. Samples are added to the plate which is then incubated and washed. The conjugate, protein G peroxidase-labelled, is added to the wells. The plate is incubated a second time at 21±3°C. After the second incubation, the plate is washed again, and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific ani-*Mycoplasma bovis* immunoglobulins are present in the test sera, plasma or milk, the conjugate remains bound to the microwell that contains the bacterial recombinant antigen, and the enzyme catalyzes the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue color is proportionate to the titre of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitized by the recombinant *M. bovis* protein. It is possible to quantify the reactivity of an unknown sample on a scale ranging from 0 to +++++.



C. Material required but not provided

- Distilled/demineralized water.
- Dilution microplates (optional).
- Graduated mono or multichannel pipettes (2-20µL, 20-200µL and 10-1000µL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer (optional).
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...

Additional kit

- **Tracer Mycoplasma bovis (Ref. : BDE K 302).** Internal reference material for *mycoplasma bovis* serology by ELISA.

D. Warnings and precautions of use

- The reagents must be kept between +2 and +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 1M 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 the 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 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 the samples

- **Serum samples** and kit controls (positive and negative control) are to be diluted **100 times** in the dilution solution and homogenized.
  Avoid using hemolyzed or coagulated samples.

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

- **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. Use undiluted skimmed milk samples in the wells.

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, 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.

1. Distribute the milk samples or the **diluted serum** and **diluted kit controls** at a rate of **100µL per well**. Cover and incubate the plate at **21±3°C** for **60±5min**.
2. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.
3. Distribute the **diluted conjugate** at a rate of **100µL per well**. Cover with a lid and incubate at **21±3°C** for **60±5min**.

4. Remove the content of the microplate. **Wash the microplate 3 times** with **300µL of washing solution per well.** Avoid the formation of bubbles in the wells between each wash.
5. Distribute **100µL** of **TMB solution** per well. Incubate at **21±3°C** for **10±1min** away from the light, without covering.
6. Distribute the **stopping solution** at a rate of **100µL per well**. Color changes from blue to yellow.
7. Record optical densities using a plate spectrophotometer with a **450nm filter** within **5 minutes** after adding the stopping solution.

H. Validation of results

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

- The difference between the optical density (OD) readings of the odd and even wells of the positive control is greater than 0,800.

  Positive control: $OD_{\text{odd well}} - OD_{\text{even well}} > 0{,}800$

- The difference between the optical density (OD) readings of the odd and even wells of the negative control is less than 0,300.

  Negative control: $OD_{\text{odd well}} - OD_{\text{even well}} < 0{,}300$

I. Interpretation of results

- Calculate for each sample its “delta OD” by subtracting the optical density from the even wells to the odd wells.
- Proceed the same way for positive and negative controls.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

$$\text{Val(ue)} = \frac{\text{Delta OD sample} * 100}{\text{Delta OD pos}}$$

By using the table below, determine the level of positive for the serums, plasmas, and milks.

	Results	Status
Sample	Val ≤ 37%	0
	37% < Val ≤ 60%	+
	60% < Val ≤ 83%	++
	83% < Val ≤ 106%	+++
	106% < Val ≤ 129%	++++
	129% < Val	+++++

A sample must be considered **positive** if it yields a result that is **greater than or equal to one plus sign (+).**

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

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

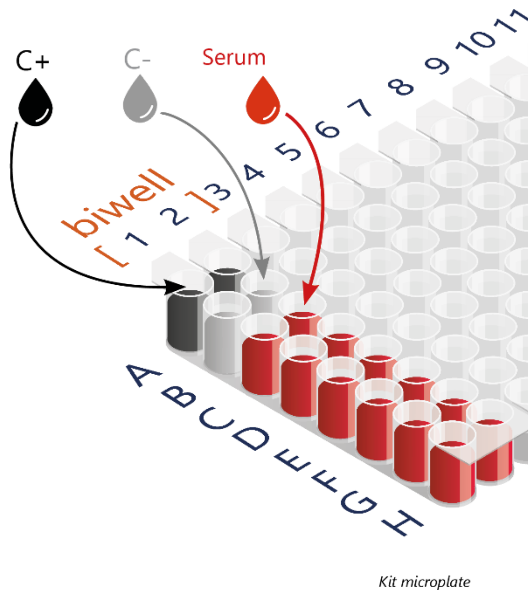
Symbols

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

Notes*

Serum protocol

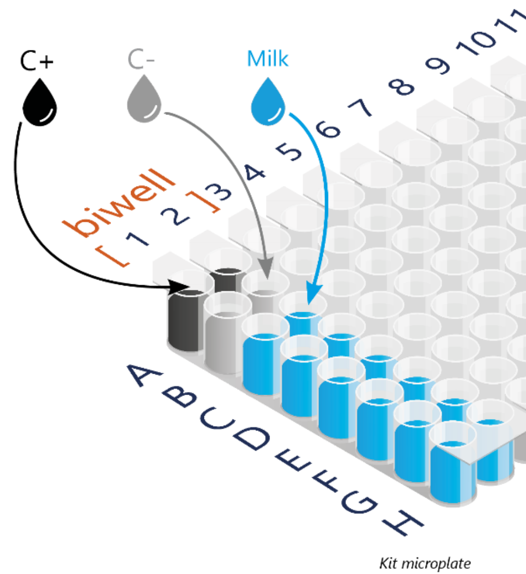
1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative control) 1/100



Kit microplate

Milk protocol

1 Distribute the centrifuged samples
Dilution of the kit controls (positive and negative contro) 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 stop solution

5 Record the optical densities

450 nm

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

BioX Diagnostics

Smart solutions for sharp decisions

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