Instructions for use
BIOK243-RESP-RPM_NO_(EN)_V03
12/06/2026

MULTISCREEN Ab ELISA

Multiscreen AgELISA Bovine respiratory

Reference : BIO K 243

ELISA kit for serodiagnosis of BRSV, BPI3 and *Mycoplasma bovis*

Biwell, indirect test

In vitro and strictly veterinary use

Sample / Dilution	Bovine
Blood sera / plasmas* / 100X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 243/2
Format	2 plates, strips of 8 wells
Reactions	48 tests

Composition of the kit

Provided material		Code	Type*	BIO K 243/2
Microplate	Microplate	D00613	1	2
Washing solution (20X)	Washing solution (20X)	D00695	A	1 X 100 mL
Dilution solution (1X)	Colored dilution solution (1X)	D01511	A	1 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)	D01476	1	1 X 0,6 mL
CTL POS	Positive control	D01535	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
12/06/2026	V03	Layout and simplification of the entire manual. Adjustment of components 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

Respiratory disorders are of major concern for bovidae, given the frequency of such infections and the high number of animals affected. These infections occur in all countries that practice intensive livestock farming in which large numbers of animals are confined to small areas. Treatment and diagnosis are both complicated due to the multifactorial character of this disease's etiology. Viruses and bacteria combined with stress due either to transport in overcrowded vans or dirty or poorly ventilated stabling, for instance, play a key role in triggering acute respiratory infections. These infections are particularly common among young animals, although they also affect adult animals.

In most cases the animals that show signs of respiratory ailments harbour several pathogens, some of which may act synergistically. So, it is generally recognised that viruses are the first pathogens to intervene, whereas bacteria act as second invaders to worsen the animal's condition. Shipping fever is a good example of the synergism that can exist between a virus (BPI3) and a bacterium, such as *Mannheimia haemolytica*, in the respiratory tract.

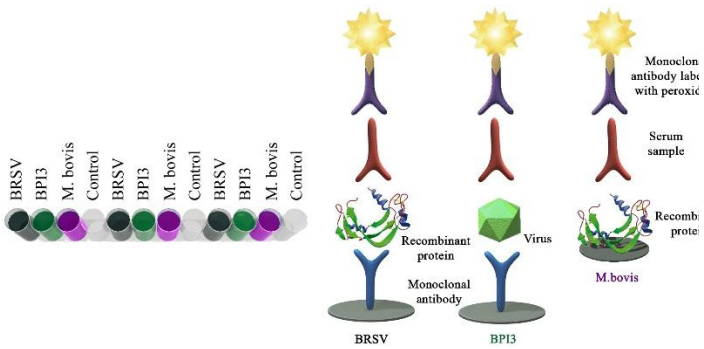
The Bio-X Respiratory trivalent ELISA kit consequently enables one to evaluate the humoral immune response of cattle to three pathogens commonly implicated in bovine respiratory infections. These are the bovine respiratory syncytial virus (BRSV, parainfluenza 3 virus (BPI3) and *Mycoplasma bovis*.

B. Test principle

The test uses 96-well microtitration plates sensitised by monoclonal antibodies specific to BRSV and PI3. These antibodies are used to trap the pathogens as well as to purify them from lysates of the cells in which the viruses were grown. The test uses a recombinant protein from *Mycoplasma bovis* expressed in *E. coli*. The distribution of these pathogens on the microtitration plate is as follows:

- Columns 1, 5 & 9: BRSV
- Columns 2, 6 & 10: BPI3
- Columns 3, 7 & 11: *Mycoplasma bovis*
- Columns 4, 8 & 12: negative control

Columns 4, 8 & 12 contain one monoclonal antibody. Using such a control reduces the number of false positives considerably. The test sera and plasma are diluted 1:100 in the dilution solution and incubated on the plate for one hour at 21°C +/- 3°C. The plate is washed and the conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, is added to the wells. The plate is incubated a second time at 21°C +/- 3°C and washed again and chromogen (tetramethylbenzidine) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific immunoglobulins are present in the test sera the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. It is possible to quantify the reactivity of an unknown sample on a scale ranging from 0 to +++++



C. Additional material and required equipment (not provided)

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

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 the dilution of samples, kit controls (positive and negative) and conjugate.
- The conjugate is to be diluted 50-fold in the dilution solution.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Blood serum samples** and kit controls must be diluted **100-fold** in the dilution solution. Avoid using haemolysed samples or those containing coagulum.

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

G. Procedure

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

1. Distribute **100 µL per well** of **diluted** samples, and kit controls. Distribute in the microplate for example as follows: positive control: wells H1 to H4, negative control: wells G1 to G4, sample 1: wells A1 to A4, sample 2: wells B1 to B4, etc.
   Cover and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.

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 well between each wash.

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

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

6. Distribute the **stop solution** at rate of **100 µL** per well. The colour 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 stop solution.

H. Validation of results

The test can only be **validated** if the positive control yields a difference on optical density at 10 minutes that is greater for each valence than:

- BRSV > 1,100
- BPI3 > 1,000
- M. bovis > 1,200

And the negative control yields a difference in optical density at 10 minutes that is lower than 0,300.

I. Interpretation of results

Subtract from each value recorded in columns 1, 2, 3 the signal of the corresponding negative control well 4 and write down the result (calculation of delta DO). To perform this calculation, take into account the possible existence of negative values. Perform the same operation for the wells corresponding to the positive control and negative control.

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.

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

Using the following table, determine each serum's, plasma's degree of positivity.

A reliable diagnosis can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2- to 3-week intervals. The first sample must be taken during the acute phase of the infection. A frank seroconversion is considered to have occurred if the signal increases by two orders of magnitude (for example, ++ -> ++++ or + -> +++).

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

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- Very easy to use

SCAN ME

Symbols

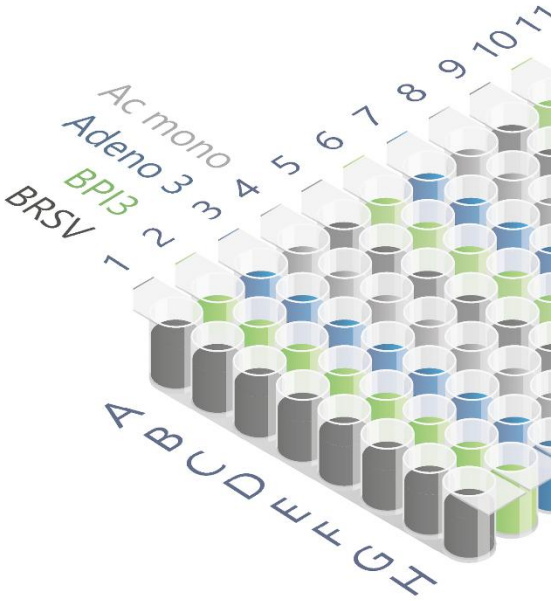
Symbol	Meaning
REF	Catalog number
	Manufacturer
	Temperature limit
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	Keep away from light
	Keep dry
	Corrosive substance
	Hazardous/irritating product

	0		+		++		+++		++++		+++++
BRSV	Val ≤	23%	< Val ≤	46%	< Val ≤	68%	< Val ≤	91%	< Val ≤	114%	< Val
BPI3	Val ≤	26%	< Val ≤	52%	< Val ≤	78%	< Val ≤	104%	< Val ≤	130%	< Val
M. bovis	Val ≤	29%	< Val ≤	47%	< Val ≤	64%	< Val ≤	82%	< Val ≤	100%	< Val

Notes*

1 Distribute 100 µL of diluted samples 1/100
& 100 µL of diluted kit controls (Ctl+ & Ctl-) 1/100

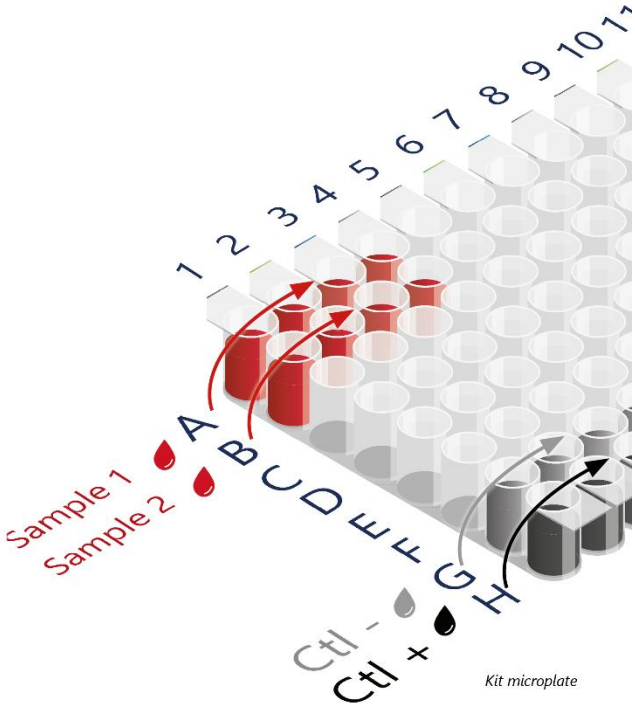
Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of conjugate 1/50

Room Temp. — 60 min → 3X Wash



Kit microplate

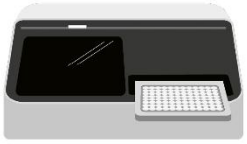
3 Add 100 µL of TMB

Room Temp. — 10 min — Obscurity

4 Add 100 µL of stop solution

5 Record optical densities

450 nm



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

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