MULTISCREEN Ab ELISA

Instructions for use
BIOK369-Respi-RPMMC_NO_(EN)_V02
03/02/2025

Multiscreen AbELISA bovine respiratory

Reference : BIO K 369

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

Biwell, indirect test

In vitro and strictly veterinary use

Sample / Dilution	Bovine
Serum - Plasma / 100X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 369/2
Format	2 plates, strip of 8 wells
Reactions	32 tests

Composition of the kit

Provided material		BIO K 369/2
Microplate	Microplates	2
Washing solution	Washing solution (20X)	1 X 100 mL
Dilution solution	Colored dilution solution (5X)	1 X 50 mL
Conjugate	Conjugate (50X)	1 X 0,5 mL
CTL POS	Positive control (1X)	1 X 0,5 mL
CTL NEG	Negative control (1X)	1 X 0,5 mL
TMB solution	Single component TMB (1X)	1 X 25 mL
Stop solution	Stop solution (1X)	1 X 25 mL

Revision history

Date	Version	Modifications
03/02/2025	V02	Layout and simplification of the entire leaflet. Replacement of Adenovirus 3 by Coronavirus. Modification of stop solution volume from 15 mL to 25 mL.

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 diseases 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 ELISA kit consequently enables one to evaluate the humoral immune response of cattle to five pathogens commonly implicated in bovine respiratory infections. These are bovine respiratory syncytial virus (BRSV), parainfluenza 3 virus (BPI3), coronavirus, *Mycoplasma bovis* and *Mannheimia haemolytica*.

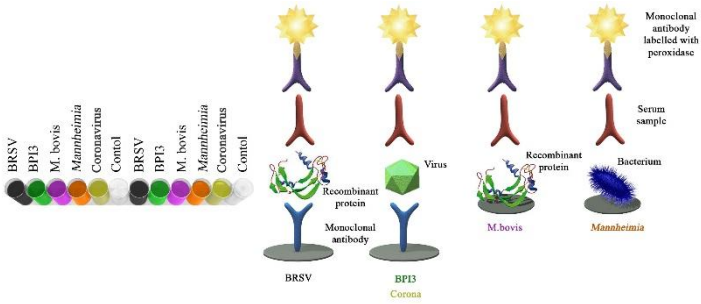
B. Test principle

The test uses 96-well microtitration plates sensitised by monoclonal antibodies specific to the three pathogens (BRSV, BPI3 and coronavirus). 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. For *Mycoplasma bovis*, the plate sensitised by a recombinant protein from *Mycoplasma bovis* expressed by *E. coli*. A gene from *Mycoplasma bovis* is expressed by this recombinant *E.coli* culture. For *Mannheimia haemolytica* the plate sensitised by purified LipoPolySaccharide (LPS).

The distribution of these pathogens on the microtitration plate is as follows:

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

Columns 6 & 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±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 reincubated at 21±3°C for 1 hour. After this second incubation, the preparation is washed 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 immunoglobulins are present in the test sera the conjugate remains bound to the corresponding microwell 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 (450nm filter).
- Microplate washer (optional).
- Dilution microplate (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.
- 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 must be diluted 5-fold in distilled/demineralized water. 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

- **Serum** samples as well as kit controls (positive and negative control) are to be diluted 100-fold in the dilution solution. Avoid using haemolysed samples or those containing coagula.

  Recommended dilution:
  990 µL dilution solution + 10 µL sample

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 **diluted** kit controls, for example as follows: positive control: wells H1 to H6, negative control: wells G1 to G6, sample 1: wells A1 to A6, samples 2: wells B1 to B6.
   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 for **10 ± 1 min** at **21 ± 3°C** 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 in optical density at 10 minutes that is greater for each valence than:

BRSV	> 1,200
BPI3	> 0,800
M. bovis	> 1,200
Mannheimia	> 1,000
Coronavirus	> 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

- Substract from each value recorded in columns corresponding to the positive and negative controls the signal of the corresponding negative control well 6 and write down the result (calculation of delta OD). Allow for any negative values that may exist in performing the calculation.
- Carry out the same operations for the columns 1, 2, 3, 4, 5.

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

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

Using the table at the bottom of the page, determine each serum's degree of positivity.

A reliable diagnosis can be made only if frank seroconversion can be documents 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 cincreases 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 (+).

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ANALYSISCREEN

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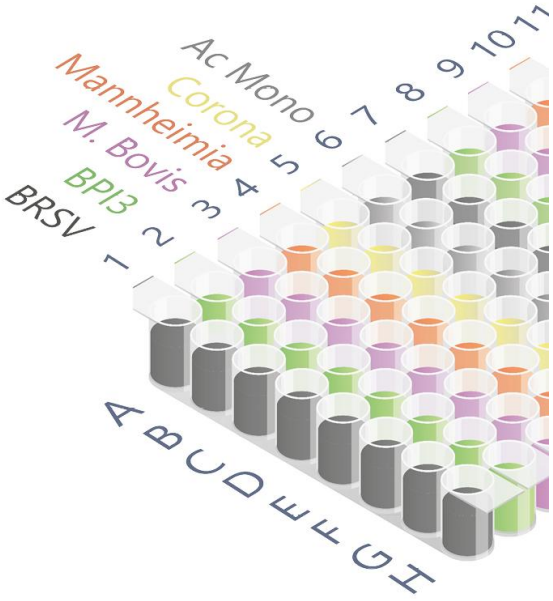
SCAN ME

	0		+		++		+++		++++		+++++
BRSV	Val ≤	20%	< Val ≤	41%	< Val ≤	62%	< Val ≤	84%	< Val ≤	105%	< Val
BPI3	Val ≤	30%	< Val ≤	59%	< Val ≤	88%	< Val ≤	116%	< Val ≤	145%	< Val
M. bovis	Val ≤	66%	< Val ≤	85%	< Val ≤	103%	< Val ≤	122%	< Val ≤	140%	< Val
Man. haemo	Val ≤	20%	< Val ≤	40%	< Val ≤	60%	< Val ≤	80%	< Val ≤	100%	< Val
Coronavirus	Val ≤	60%	< Val ≤	70%	< Val ≤	80%	< Val ≤	90%	< Val ≤	100%	< Val

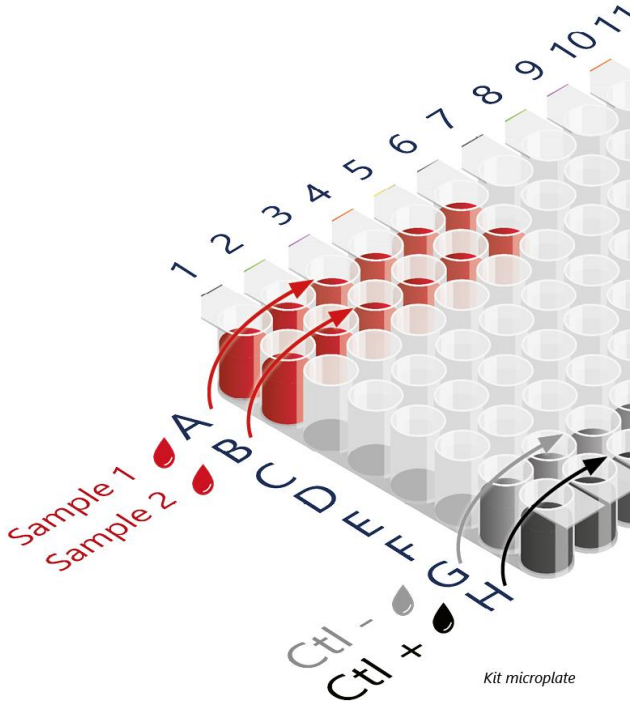
Notes*

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



Kit microplate

2 Add 100 µL of conjugate 1/50



Kit microplate

3 Add 100 µL of TMB

4 Add 100 µL of stop solution

5 Record optical densities

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

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