MONOSCREEN Ab ELISA

Instruction manual
BIOK061-BRSV_NO_(EN)_V03
30/04/2024

Monoscreen AbELISA BRSV

Reference : BIO K 061

ELISA test for serodiagnosis of Bovine Respiratory Syncytial Virus (BRSV)
Biwell, indirect test

For veterinary *in vitro* use only

Sample / dilution	Bovine
Serum – plasma* / 100X	✓
Milk / 4X	✓

*Hereafter, we will refer to it as serum.

Presentation

Product reference	BIO K 061/2	BIO K 061/5
Format	2 plates, strips of 16 wells	5 plates, strips of 16 wells
Reactions	96 tests	240 tests

Kit composition

Provided material		BIO K 061/2	BIO K 061/5
Microplate	Microplates	2	5
Washing solution	Washing solution (20X)	1 x 100 mL	1 x 250 mL
Dilution solution	Colored dilution solution (5X)	1 x 50 mL	1 x 100 mL
TMB solution	TMB Solution (1X)	1 x 25 mL	1 x 55 mL
Stop solution	Stopping solution (1X)	1 x 15 mL	1 x 30 mL
Conjugate	Conjugate (50X)	1 x 0,5 mL	1 x 1,4 mL
CTL POS	Positive control	1 x 0,5 mL	1 x 0,5 mL
CTL NEG	Negative control	1 x 0,5 mL	1 x 0,5 mL
Tracer	Tracer	1 x 0,5 mL	1 x 0,5 mL

Revision history

Date	Version	Modifications
30/04/2024	V03	Layout modification and simplification of the entire instruction manual

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

A. Introduction

In Europe, BRSV is the most important etiological agent responsible for respiratory affections in young cattle. In cattle as in children, respiratory syncytial viruses can cause a very deep attack of the respiratory tree. The affection id often provoking very severe injuries, which are responsible for important economic losses. As a matter of fact, in Europe, 7 million calves suffer from infectious diseases yearly, 60% of which are caused by respiratory pathogens. One million calves are dying each year of respiratory diseases in the European Community. The cost of these diseases which includes medical treatments, growth delays and mortality is about 450 million EURO per year for calves under one year. For dairy cattle, the cost of BRSV has been evaluated at around 25 EURO per animal.

BRSV is principally affecting young cattle. Beef cattle is especially vulnerable because of the muscular mass of the animal which is very important compared to the pulmonary volume. Clinical manifestations can be dramatic. Often signs of severe pneumonia such as polypnea, abdominal breathing and hyperthermia are present. Reinfections can be observed but most often they remain subclinical. Clinical diagnosis is very difficult and laboratory assistance is required for a precise diagnosis. Virus can be detected in lung tissue by fluorescein labelled specific antibodies.

Diagnosis can also be achieved by measuring a virus specific seroconversion. To do so, a first sample will be collected during the acute phase of the disease and a second sample will be collected 2 or 3 weeks later. These two samples will be evaluated for their content in specific antibodies against BRSV by ELISA.

B. Test principle

The test uses 96-well microtitration plates sensitized by monoclonal antibodies specific to F protein of BRSV virus. This antibody is used to trap a recombinant F protein. The plate's odd columns (1, 3, 5, 7, 9, 11) contain the recombinant viral protein, whereas the even columns (2, 4, 6, 8, 10, 12) contain a control antigen. We thus have a genuine negative control to differentiate the specific anti-viral antibody from the antibodies directed against the control antigen. Using such a control reduces the number of false positives considerably.

The test blood sera or milks are diluted in the dilution solution. Samples are added to the plate which is then incubated and washed. The conjugate, a peroxidase-labelled anti-bovine IgG1 monoclonal antibody, is added to the wells. The plate is then incubated a second time at 21±3°C and 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 BRSV immunoglobulins are present in the test sera the conjugate remains bound to the microwell that contains the viral antigen, and the enzyme catalyzes the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of the specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitized by the viral antigen. 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.
- 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,...

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.
- Do not use reagents from other kits.
- 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 must be diluted 5 times in distilled/demineralized water. The dilution is colored in yellow. It is used for dilution of samples, kit controls (positive and negative controls and tracer), 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.
- The tracer is a reference sample that can be used to check the intra-laboratory reproducibility of the kit's batch. It must be diluted 100 times in the dilution solution.

F. Preparation of the samples

- **Serum samples** and kit controls (positive and negative control, and tracer) 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 minutes at 4000g**. Take up the middle layer of liquid taking care not to touch the underlying cell sediment. Samples are to be diluted **4 times** in the dilution solution and homogenized.

  Recommended dilution:
  250 µL of sample + 750µL of dilution solution.

G. Procedure

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

1. Distribute the **diluted samples** 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** in the dark without covering.
6. Distribute the **stopping solution** at a rate of **50µL 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 each value obtained by the corresponding value obtained with the positive control and multiply this result by 100 to express it as a percentage.

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

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

	Results	Status
Sample	Val ≤ 20%	0
	20% < Val ≤ 40%	+
	40% < Val ≤ 60%	++
	60% < Val ≤ 80%	+++
	80% < Val ≤ 100%	++++
	Val > 100%	+++++

A reliable diagnostic 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 (two plusses; for example, ++ → ++++ or + → +++).

A sample must be considered **positive** if the result is **greater or equal to one plus sign**.

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ANALYSISCREEN

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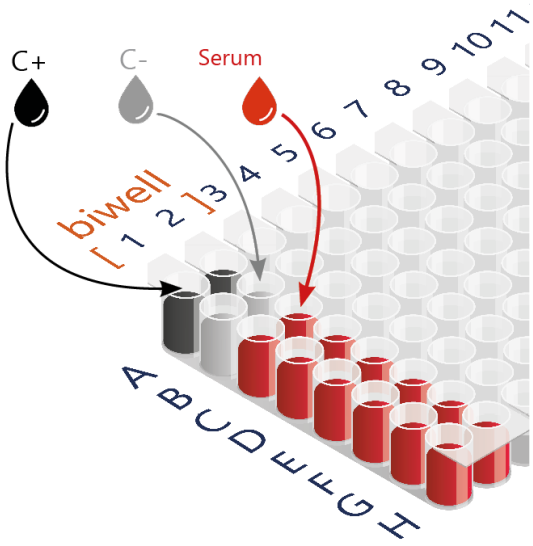
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

Notes*

Serum protocol

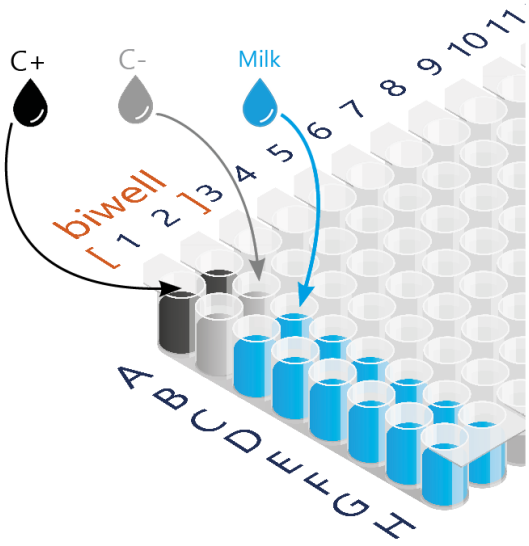
1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative control and tracer) 1/100



Kit microplate

Milk protocol

1 Dilution of samples 1/4
Dilution of the kit controls (positive and negative control and tracer) 1/100



Kit microplate

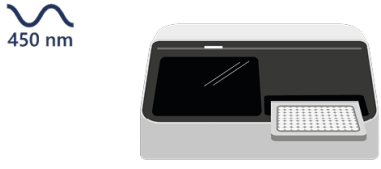
Joint protocol

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

3 Add 100 µL of TMB

4 Add 50 µL of stop solution

5 Record the optical densities



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

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