



MONOSCREEN[®] Ab ELISA

Instruction manual
 BIOK061-BRSV_NO_(EN)_V04
 26/06/2026

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		Type*	Code	BIO K 061/2	Code	BIO K 061/5
Microplate	Microplates	1	D00482	2	D00483	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	3 x 125 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	D01476	1 x 0,6 mL	D01477	1 x 1,5 mL
CTL POS	Positive control	a	D01501	1 x 0,5 mL	D01501	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
26/06/2026	V04	Layout modification and simplification of the entire instruction manual. Adjustment of components 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

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 is 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 $21\pm 3^{\circ}\text{C}$ 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 (2-20 μL , 20-200 μL and 10-1000 μL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer (optional).
- Dilution microplate (optional).
- Incubator at $21\pm 3^{\circ}\text{C}$.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...

Additional kit

- **Tracer BRSV (Ref. : BDE K 061)**. Internal reference material for bovine respiratory syncytial virus serology by ELISA.

D. Warnings and precautions of use

- The reagents must be kept between $+2$ and $+8^{\circ}\text{C}$.
- 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 $21\pm 3^{\circ}\text{C}$ 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 is colored in yellow. It is used for dilution of samples, kit controls (positive and negative 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:

10 μL of sample + 990 μL 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 + 750 μL of dilution solution.

G. Procedure

- Bring all the reagents to $21\pm 3^{\circ}\text{C}$ 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 **diluted samples** and **diluted kit controls** at a rate of **100 μL per well**. Cover and incubate the plate at **$21\pm 3^{\circ}\text{C}$ for 60 \pm 5min**.
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 between each wash.
3. Distribute the **diluted conjugate** at a rate of **100 μL per well**. Cover and incubate at **$21\pm 3^{\circ}\text{C}$ for 60 \pm 5min**.

- 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.
- Distribute **100µL of TMB solution** per well. Incubate at **21±3°C for 10±1min** in the dark without covering.
- Distribute the **stopping solution** at a rate of **100µL per well.** Color changes from blue to yellow.
- 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	$\text{Val} \leq 20\%$	0
	$20\% < \text{Val} \leq 40\%$	+
	$40\% < \text{Val} \leq 60\%$	++
	$60\% < \text{Val} \leq 80\%$	+++
	$80\% < \text{Val} \leq 100\%$	++++
	$\text{Val} > 100\%$	+++++

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**.

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



AnalysisScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysisScreen™** 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



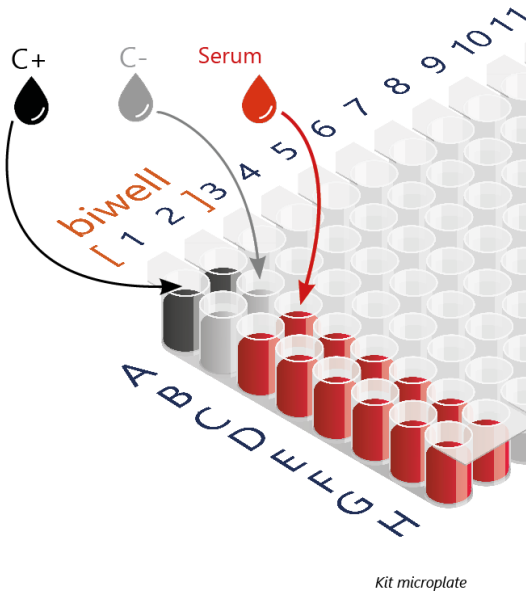
SCAN ME

Symbols

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

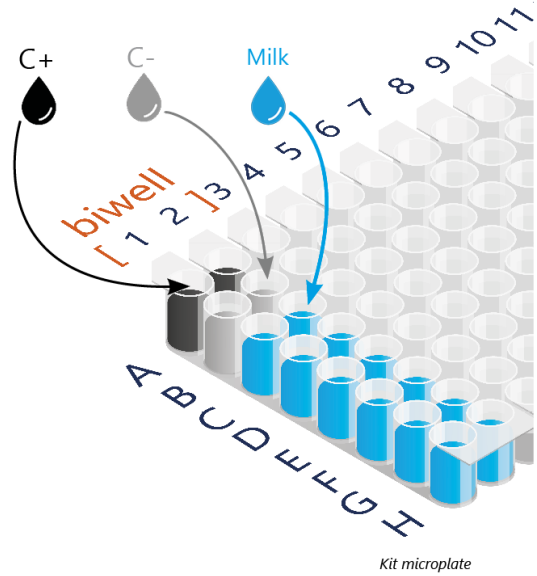
Serum protocol

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



Milk protocol

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



Joint protocol

- 2 Add 100 µL of diluted conjugate



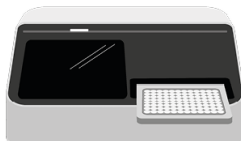
- 3 Add 100 µL of TMB



- 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.