MONOSCREEN Ab ELISA

Instruction manual
BIOK239-BPI3_NO_(EN)_V03
02/06/2026

Monoscreen AbELISA BPI3

Reference : BIO K 239

ELISA test for serodiagnosis of Bovine Parainfluenza 3 Virus (BPI3)
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 239/2
Format	2 plates, strips of 16 wells
Reactions	96 tests

Kit composition

Provided material		Code	Type*	BIO K 239/2
Microplate	Microplates	D00587	1	2
Washing solution (20X)	Washing solution (20X)	D00695	A	1 x 100 mL
Dilution solution (1X)	Colored dilution solution (1X)	D01511	A	2 x 125 mL
TMB solution (1X)	Single component TMB (1X)	D01594	B	1 x 30 mL
Stop solution (1X)	Stopping solution (1X)	D00680	A	1 x 30 mL
Conjugate (50X)	Conjugate (50X)	D01596	1	1 x 0,6 mL
CTL POS	Positive control	D01458	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
02/06/2026	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

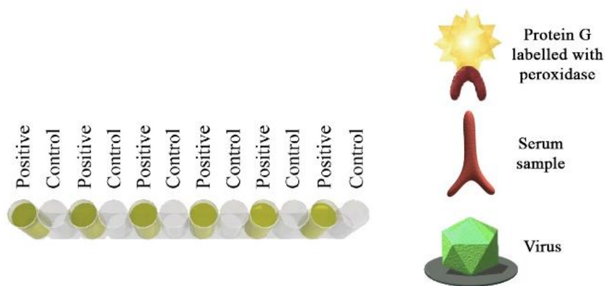
Parainfluenza 3 was first isolated in the USA from the nasal mucus of cattle showing clinical signs of shipping fever. Its distribution in the cattle has been found to be worldwide. Most reports of bovine PI3 virus activity have been in groups of young cattle with respiratory diseases such as enzootic calf pneumonia and shipping fever. Bovine PI3 virus infections are not invariably associated with disease, and subclinical infections often occur. In European countries, BPI3 infection mostly occurs during the months from October to March. BPI3 virus infection may be accompanied by concurrent infection of the respiratory tract by other viruses such as respiratory syncytial virus, adenovirus or BVDV. In outbreaks of bovine respiratory disease, it is not possible to diagnose BPI3 virus infection on clinical grounds alone. To establish a diagnosis, it is necessary to take paired sera from infected animals or to submit animals from the outbreak for necropsy to facilitate immunocytochemical examinations of the lower respiratory tract. BPI3 virus infection in an outbreak of respiratory disease can be detected by the demonstration of a rise in serum antibody titer to the virus between acute and convalescent phase serum samples (seroconversion).

B. Test principle

The test uses 96-well microtitration plates sensitized by purified BPI3 virus. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the virus, whereas the even columns (2, 4, 6, 8, 10 and 12) contain a control antigen.

The test blood sera and plasma are diluted in the dilution solution. The milks 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°C +/- 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 PI3 immunoglobulins are present in the test sera or milk 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 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 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 (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 BPI3 (Ref.: BDE K 239):** Internal reference material for BPI3 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 (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:
  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 (100µL) into the test microplate using a multi-channel pipette.

1. Distribute the **diluted serum** samples, and **undiluted milk** 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 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 samples.

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

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 it yields a result that is **greater than or equal to one plus sign**.

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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

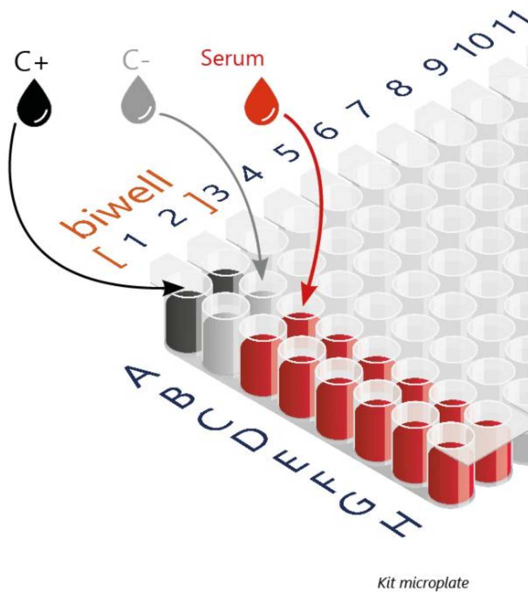
Symbols

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

Notes*

Serum protocol

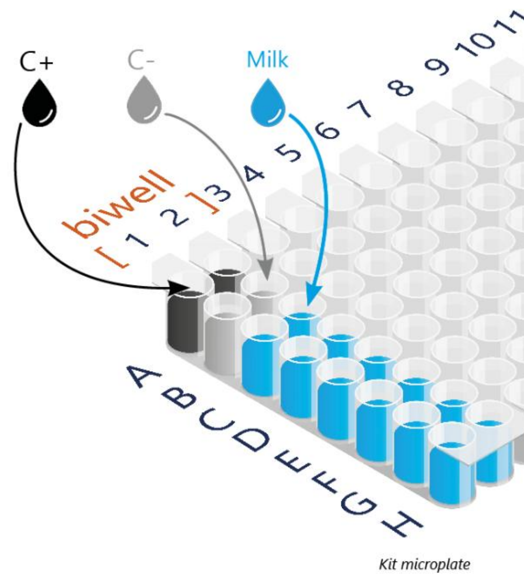
1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative control) 1/100



Kit microplate

Milk protocol

1 Distribute the centrifuged samples
Dilution of the kit controls (positive and negative control) 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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