MONOSCREEN Ab ELISA

Instruction manual
BIOK063-ADN3_NO_(EN)_V03
26/04/2024

Monoscreen AbELISA Bovine Adenovirus 3

Reference : BIO K 063

ELISA test for serodiagnosis of Bovine Adenovirus 3

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 063/2
Format	2 plates, strips of 16 wells
Reactions	96 tests

Kit composition

Provided material		BIO K 063/2
Microplate	Microplates	2
Washing solution	Washing solution (20X)	1 x 100 mL
Dilution solution	Colored dilution solution (5X)	1 x 50 mL
TMB solution	TMB Solution (1X)	1 x 25 mL
Stop solution	Stopping solution (1X)	1 x 15 mL
Conjugate	Conjugate (50X)	1 x 0,5 mL
CTL POS	Positive control	1 x 0,5 mL
CTL NEG	Negative control	1 x 0,5 mL
Tracer	Tracer	1 x 0,5 mL

Revision history

Date	Version	Modifications
26/04/2024	V03	Layout and simplification of the entire manual

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

A. Introduction

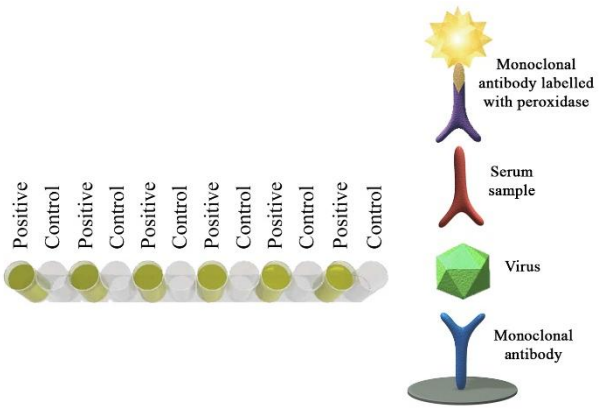
Bovine adenoviruses (BAV) are classified into nine serotypes (species) so far. These nine serotypes are divided into two subgroups mainly on the basis of replication in either calf kidney or testicle cells (subgroup I, serotypes 1-3 and 9) or only in testicle cells (subgroup II, serotypes 4-8).

The two subgroups also differ in their antigenic properties. The distribution of BAV in cattle populations is worldwide. Serological evidence suggests a high incidence of infection. Adenoviruses are generally present in large herds. Viruses are excreted by different forms of shedding and by couching. Infection occurs most frequently when calves are 3 weeks to 4 months old. In diseased calves respiratory and enteric symptoms, fever and anorexia can be observed. The disease usually starts with respiratory symptoms. Serous excretions from the nose and conjunctivae are accompanied by coughing. Enteric infection becomes manifest by salivation and thin, greyish-yellow feces. The disease can be aggravated by secondary bacterial infections. For the diagnosis of the disease, paired serum samples should be taken from calves and tested by ELISA method. If no acute disease is observed but infection of the herd is suspected, a serological survey can be performed. In this case, single serum samples are collected from adult animals representing 5-10% of the herd. Bio-X bovine adenovirus 3 ELISA kit is subgroup-specific and can only detect immune response against subgroup I bovine adenovirus.

B. Test principle

The test uses 96-well microtitration plates sensitized by monoclonal antibodies specific to one of the antigenic determinants of bovine adenovirus 3. This antibody is used to trap the virus as well as to purify it from lysate of the cells in which the virus was grown. The plate's odd columns (1, 3, 5, 7, 9, 11) contain the virus, whereas the even columns (2, 4, 6, 8, 10, 12) contain a lysate of bovine kidney cell line that was used as a substrate to propagate the virus. We thus have a genuine negative control to differentiate the specific anti-viral antibody from the antibodies directed against the antigenic determinants of the bovine kidney cells used for its replication. Using such a control reduces the number of false positives considerably.

The test blood sera or milks are diluted in the dilution solution. The plate is incubated and washed, then 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 advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific bovine adenovirus 3 immunoglobulins are present in the samples, 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 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 serum 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,...

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 is to be diluted 5 times in distilled/demineralized water. The dilution solution is colored in yellow. It is used for dilution of samples, kit controls (positive and negative control 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 serum 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. Milk 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** away from the light, 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 0,600.

  Positive control: $OD_{\text{odd well}} - OD_{\text{even well}} > 0{,}600$

- The difference between the optical density (OD) readings of the odd and even wells of the negative control is less than 0,200.

  Negative control: $OD_{\text{odd well}} - OD_{\text{even well}} < 0{,}200$

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.

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

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

	Results	Status
Sample	Val ≤ 10%	0
	10% < Val ≤ 33%	+
	33% < Val ≤ 56%	++
	56% < Val ≤ 79%	+++
	79% < Val ≤ 102%	++++
	Val > 102%	+++++

A reliable diagnostic can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2 to 3 weeks 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 crosses; for example, ++ → ++++ or + → +++).
A sample must be considered **positive** if the result is **greater or equal to one cross**.

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ANALYSISCREEN

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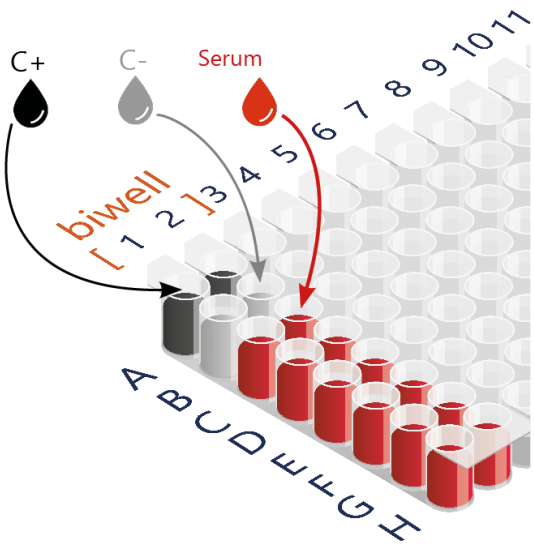
SCAN ME

Notes*

Serum protocol

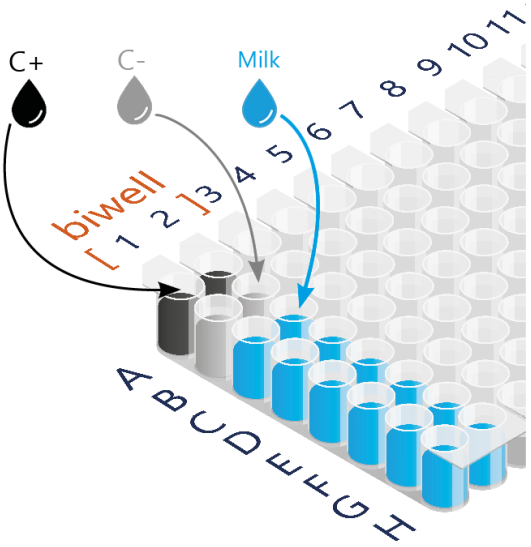
1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative control and tracer) 1/100



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

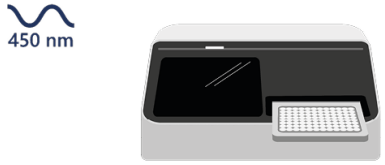
Joint protocol

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.

Contact us
support.immuno@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)