Instruction manual
BIO K 126-Rota sero _NO_(EN)_V04
27/03/23

MONOSCREEN Ab ELISA

Monoscreen AbELISA *Rotavirus* bovine

Reference: BIO K 126

Competitive ELISA test for serological diagnostic of bovine rotavirus

For veterinary *in vitro* use only

Sample	Species	Individual analysis
Serum	Bovine	✓

* This is done in accordance with the legislation in force in your country, the certifying body or the recommendations made by the NRL when they exist.

Presentation

Product reference	BIO K 126/2
Format	2 plates, strip of 8 wells
Reactions	192 tests

Composition of the kit

Provided material	BIO K 126/2
Microplates	2
Washing solution (20X)	1 X 100 mL
Colored dilution solution (1X)	1 X 100 mL
Conjugate (50X)	1 X 0,55 mL
Positive control	1 X 0,5 mL
Negative control	1 X 0,5 mL
Single component TMB (1X)	1 X 25 mL
Stop solution (1X)	1 X 15 mL

Revision history

Date	Version	Modifications
27/03/2023	V04	Layout modification and simplification of the entire instruction manual. Removal of the colostrum and milk matrices. Modification of the dilution of the conjugate.

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

A. Introduction

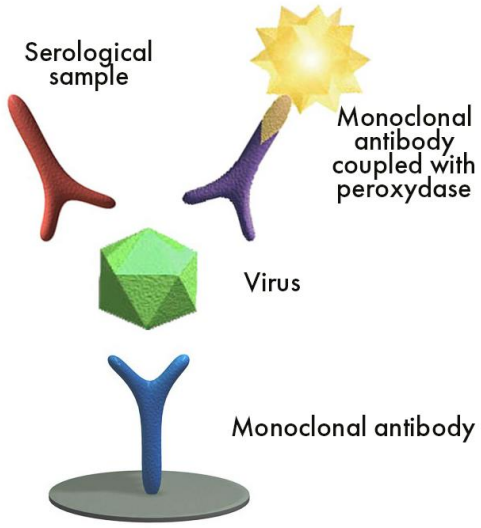
Diarrhea is one of the leading causes of death in young calves under one month old. Since Mebus's 1969 discovery that viruses could be detected in the faeces of calves with diarrhea, it has been proven that rotavirus can infect the calve and cause sometimes severe diarrhea. Rotavirus is one of the pathogens associated with gastroenteritis in young calves. Rotavirus is ubiquitous. As a result, most of the animals coming from intensive livestock farms have specific antibodies against this pathogen. The antibodies produced by the cow in response to natural immunization or vaccination are transmitted to her calf at birth via the colostrum. The colostrum immunoglobulins frequently are not transmitted to the calves correctly (poor quality colostrum, late administration, too small an amount, pre-calving mastitis, etc.). As a result, the calf will be insufficiently protected from infection. The rotavirus ELISA kit enables one to measure the suckling calf's specific protection against rotavirus.

For this, a serum sample must be taken in the first few days after birth when the calf is still protected by the colostrum and has not yet developed active immunity against the virus. However, you must wait at least 24 hours after the first dose of colostrum before taking the control blood sample to allow intestinal resorption of the immunoglobulins to take place. The kit may also be used to test the efficacy of vaccines.

B. Test Principal

The test uses 96-well microtitration plates sensitized by a rotavirus specific monoclonal antibody. This antibody was used to capture the rotavirus in order to present it for competitive testing. The samples and standard are added to the wells of the microplate at the same time as the conjugate. After incubation and washing of the preparation, the substrate solution (single component TMB) is added. The staining intensity is inversely proportional to the immunoglobulin concentration in the sample. Reading is performed at 450 nm.

This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the color is inversely proportionate to the sample's serum titer. Positive and negative control sera are provided with the kit to be able to validate the test results.



C. Material required but not provided

- Distilled/demineralized water.
- Graduated mono- or multichannel pipettes (2-20µL, 20-200µL et 100-1000µL range) and single-use tips.
- Microplate reader (450nm filter).
- Microplate washer.
- Incubator at 21±3°C.
- Standard laboratory equipment: graduated cylinder, tube rack, lid,...

D. Warnings and precautions

- 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 is ready to use. The dilution solution is colored in yellow. It is used for dilution of samples, positive and negative serums, 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 samples

- **Serum** samples and kit controls (positive and negative) should be diluted **20 times** in the dilution solution and homogenized.
  Avoid using hemolyzed or coagulated samples.

  Recommended dilution :
  10 µL of sample + 190 µL 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 **kit controls** at a rate of **100 µL per well**.

   Quickly add **100 µL** of **diluted conjugate** per well. Homogenize samples/controls and conjugate by aspiration/reflux.

   Cover with a lid 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 wells between each wash.

3. Distribute **100 µL of TMB solution** per well. Incubate the plate at **21 ± 3°C** during **10 ± 1 min** away from the light, without covering.

4. Distribute the **stopping solution** at rate of **50 µL per well**. Color changes from blue to yellow.

5. **Record the optical densities** using a plate spectrophotometer with a **450 nm filter within 5 minutes** after adding the stopping solution.

H. Validation of results

The test can only be validated if :

- The difference between positive and negative serum optical density readings is greater than 0,700.

$$OD_{\text{negative serum}} - OD_{\text{positive serum}} > 0{,}700$$

- The positive serum's inhibition percentage (%inh) is greater than 50%.

$$\%inh_{\text{positive serum}} > 50\%$$

I. Results interpretation

Calculate for each sample its inhibition percentage (%inh) using the following formula :

$$\%\ inh = \frac{OD_{\text{negative serum}} - OD_{\text{sample}}}{OD_{\text{negative serum}}} * 100$$

	Results	Status
Sample	%inh < 20%	Negative
	%inh ≥ 20 %	Positive

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

ANALYSISCREEN

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

Notes*

1 Distribute 100µL of the diluted samples (1/20) and 100µL of the diluted controls (1/20)

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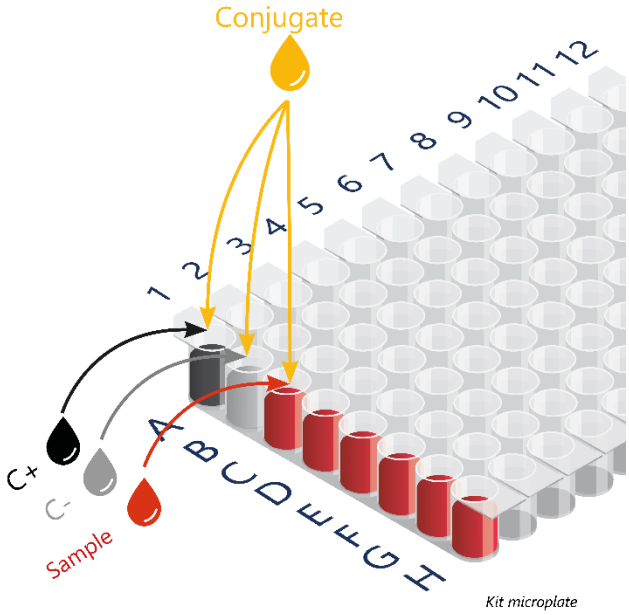
Quickly add 100 µL of diluted conjugate

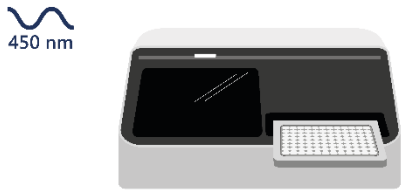


Kit microplate

2 Distribute 100 µL of TMB solution

3 Add 50 µL of stopping solution

4 Record optical densities



* Notes do not replace the instructions of use of which they are a summary

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