Instructions for use
BIOK151-Easy-Digest4_NO_(EN)_V04
14/02/2025

MULTISCREEN Ag ELISA

Multiscreen AgELISA Easy Digest 4

Reference : BIO K 151

ELISA kit for antigenic diagnostic of Rotavirus, Coronavirus, *E. coli* F5 attachment factor and *Cryptosporidium parvum*

Monowell, sandwich

In vitro and strictly veterinary use

Sample / Dilution	Bovine
Faeces / 2X	✓

Presentation

Product reference	BIO K 151/1	BIO K 151/2
Format	1 plate, strips of 8 wells Strips divisible into single wells	2 plates, strip of 8 wells Strips divisible into single wells
Reactions	96 tests	192 tests

Composition of the kit

Provided material		BIO K 151/1	BIO K 151/2
Microplate	Microplate	1	2
Washing solution	Washing solution (20X)	1 x 100 mL	1 x 100 mL
Dilution solution	Colored dilution solution (5X)	1 x 50 mL	1 x 50 mL
Conjugate	Conjugates (1X)	4 x 12 mL	4 x 12 mL
CTL POS	Positive control (1X)	1 x 3 mL	2 x 3 mL
CTL NEG	Negative control (1X)	1 x 3 mL	2 x 3 mL
TMB solution	Single component TMB (1X)	1 x 12 mL	1 x 25 mL
Stop solution	Stop solution (1X)	1 x 6 mL	1 x 15 mL

Revision history

Date	Version	Modifications
24/09/2024	V03	Layout and simplification of the entire manual.
14/02/2025	V04	Modification of the composition of CTL POS & NEG from 1x3 mL to 2x3 mL.

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

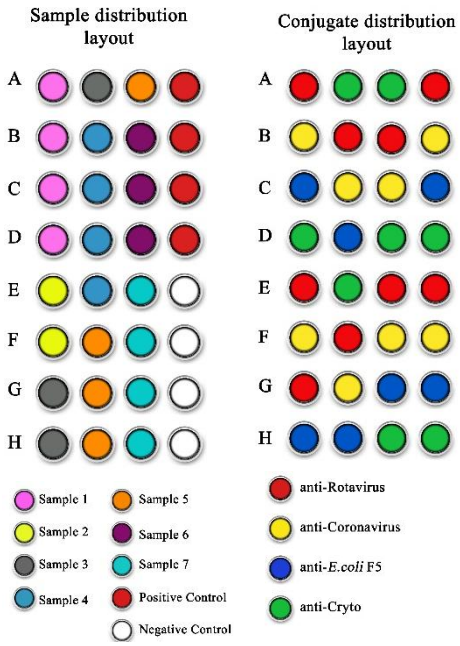
A. Introduction

Diarrhea is a major cause of mortality in calves in the first month of life. Bovine neonatalgastroenteritis is often a multifactorial disease. It can be caused by viruses (Coronavirus or Rotavirus), bacteria (*Salmonella* or *E. coli* F5), or protozoan microorganisms such as Cryptosporidum parvum. The diagnosis of the aetiological agent of diarrhea can be made only in the laboratory because it is not possible to differentiate between these different mircoorganisms on the basis of the clinical signs. The ELISA technique is simple to use, requires little in the way of equipment and is particularly well suited to analysing large numbers of samples. The test is quick and reliable and can be evaluated by the naked eye if spectrophotometric equipment is not available.

B. Test principle

In the test, the entire microtitration plate is sensitized with a mixture of antibodies that are specific for the four pathogens (see the diagram on the last page). These antibodies capture the corresponding pathogens in the faecal samples. The faecal material is diluted in the dilution solution and incubated on the microplate for ½ hour at 21±3°C. positive and negative controls are also deposited on the plate.

The plate is incubated and washed and then ready-to-use conjugates are added to the wells. The choice of conjugates is left up to the user. The diagram below gives an example of the arrangement of samples and conjugates on the plate.
Following a second incubation for ½ hour at 21±3°C, the plate is washed again and the chromogen tetramethylbenzidine (TMB), is added. This chromogen has the two advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If one or more of the pathogens being sought is present in the faeces, the corresponding conjugate or conjugates remain bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a blue compound. The intensity of the resulting colour is proportionate to the titre of the pathogen in the sample.



C. Additional material and required equipment (not provided)

- Distilled/demineralized water.
- Graduated mono- or multichannel pipettes (2-20 µL, 20-200 µL et 100-1000 µL 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. Precautions for use

- 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 must be diluted 5-fold in distilled/demineralized water.
- The conjugates are ready to use.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Faeces samples** must be diluted **2-fold** in the dilution solution.

***N.B** : This is a qualitative dilution only, which must allow the pipetting of faecal suspensions. Discard any gruds by natural decantation for about 10 minutes. Do not centrifuge the suspensions.*

G. Procedure

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

1. Distribute **100 µL per well** of **diluted** samples, and **ready to use** kit controls. Cover and incubate the plate at **21±3°C** during **30±5 min**.

***N.B.** : The arrangement of samples on the plate must be set by the user according to the number of faecal samples to test and the valences selected for each sample. For kit controls, distribute one well per valence tested.*

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 well between each wash.

3. Add **100 µL ready to use conjugates** per well.
Cover and incubate the plate **at 21±3°C** during **30±5 min**.

4. 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 well between each wash.

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

6. Distribute the **stop solution** at rate of **50 µL** per well. The colour changes from blue to yellow.

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

H. Validation of results

The test can only be **validated** if the positive control antigens yields difference in the optical densities at 10 minutes that are greater than the values:

Rotavirus	>1,000
Coronavirus	>1,000
E. coli F5	>1,000
Cryptosporidium parvum	>1,000

I. Interpretation of results

Calculate the net optical density of each sample by subtracting them from the reading for each sample well the optical density of the corresponding negative control.
Proceed in the same way for the positive control.

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.

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

Using the following table, determine each sample's status (positive, negative).

Rotavirus	Val ≥ 6%
Coronavirus	Val ≥ 7%
E. coli F5	Val ≥ 6%
Cryptosporidium parvum	Val ≥ 6%

Any sample that yields a difference in optical density that is greater or equal than the percentages above is considered positive for the valence in question.

Conversely, any sample that yields a difference in the optical density that is less than the percentage above is considered negative for the valence in question.

If the results are interpreted visually (reading of the blue colour), the samples that produce a more intense blue colour than the colour in the corresponding negative control wells are condisered to be positive.

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

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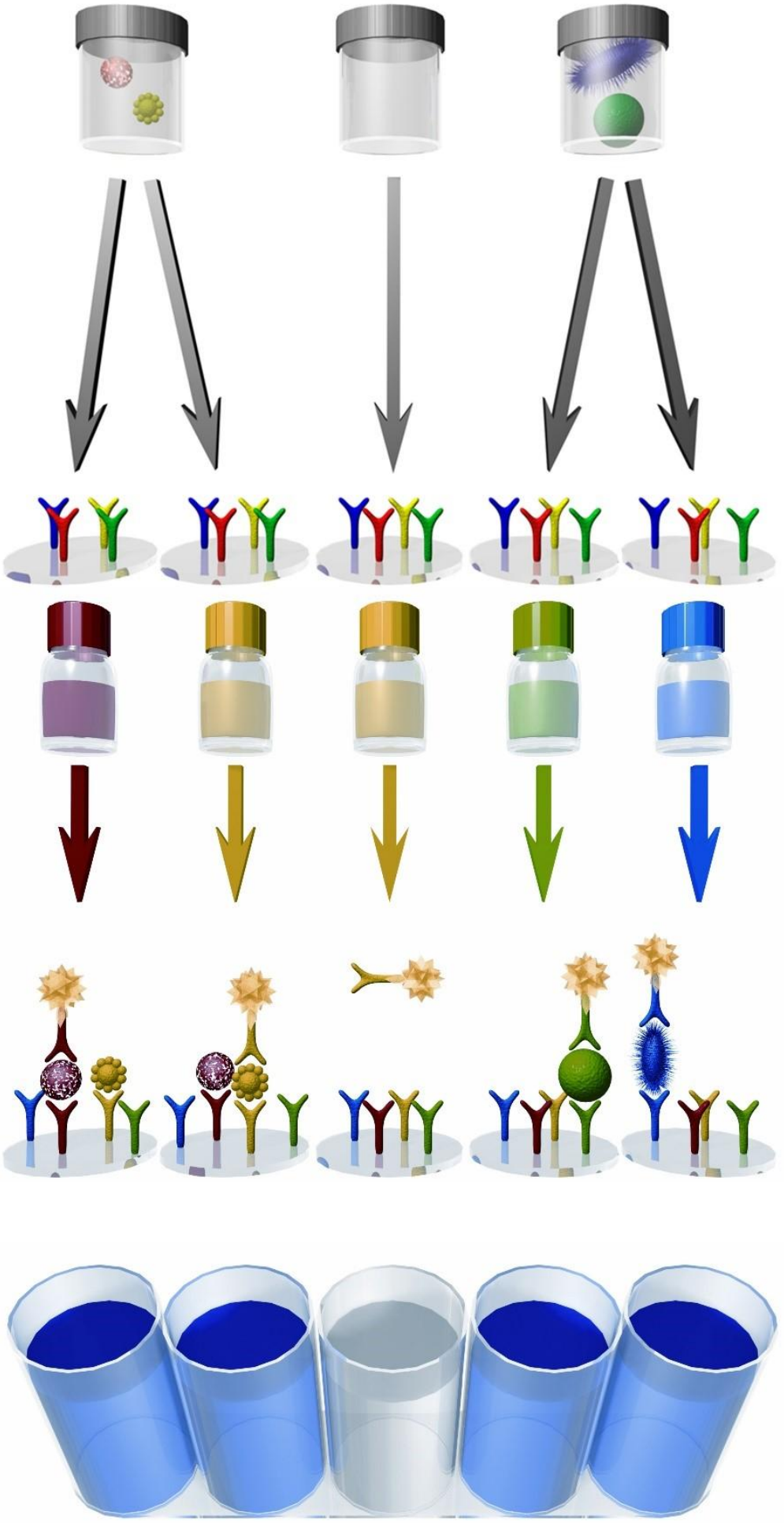
SCAN ME



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