EN

MONOSCREEN AbELISA

User Manual

BIO K 447 NO (EN) V1.1
14-07-2020

MonoScreen AbELISA *Ascaris suum*

Reference : BIO K 447

ELISA Kit for serodiagnosis of infestation caused by *Ascaris suum*

Monowell, indirect test

For veterinary *in vitro* use only

Sample	Species
Blood serum	Pig

To place an order

Product Reference	BIO K 447/2
Format	2 plates, 12X8-well strips
Reagents	2 X 96 tests

Composition of kit

	BIO K 447/2
Microplates	2
Washing Solution (20X)	1 X 100ml
Colored dilution buffer (5X)	1 X 50ml
Conjugate (50X)	1 X 0,5ml
Positive serum	1 X 0,5ml
Negative serum	1 X 0,5ml
Single Component TMB Solution (1X)	1 X 25ml
Stop Solution (1X)	1 X 15ml

Revision history

14/07/2020 - V1.1 text formatting

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

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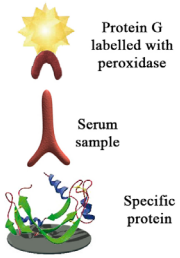
A. Introduction

Ascaris suum is a nematode worm that infects pigs. It is spread worldwide and despite the availability of effective anthelmintics, it is still very prevalent. It can be present in groups of animals of different ages. Most of the time, ascariasis is subclinical even if in the most serious cases, respiratory problems can develop following the migration of the larvae into the pulmonary parenchyma.

Ascariasis is responsible for significant economic losses for pig farmers (stunted growth, bad feed conversion, liver damage - white spots and poor carcass quality). The diagnosis of ascaris can be performed post mortem (presence of the worm in the small intestine, white spots on the surface of the liver) or *in vivo* by detecting eggs or worms in the faeces. This approach is, however, not very sensitive because few L4 larvae present in the small intestine evolve into adult stages. The detection of antibodies specific to the parasite's hemoglobin with an ELISA test is a much more sensitive technique and, if it is performed at the end of the fattening period, it is possible to estimate the infestation intensity of the pig group. This approach also allows the farmer to adapt his deworming strategy for the following groups.

B. Test Principle

The test uses 96-well microtitration plates sensitised by a specific protein from *Ascaris suum*. The entire surface of each microplate has been sensitised with the specific protein
The blood sera are diluted in the dilution buffer. 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 ± 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 anti-*Ascaris suum* immunoglobulins are present in the sera the conjugate remains bound to the microwell that contains the *Ascaris suum* protein and the enzyme catalyses 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.



C. Additional Materials and equipment required but not provided

- Distilled/demineralised water
- Single or multi-channel pipette with accuracy (range 2-20µl, 20-200µl and 100-1000µl) and disposable tips
- Microplate Reader (filter 450nm)
- Microplate washer and shaker (optional)
- Dilution Microplate
- Standard laboratory material : graduated cylinder, tube holder, lid,...

D. Precautions for use

- Store reagents between +2 and + 8 ° C. The washing solution can be stored at room temperature.
- Keep unused strips in the sealed aluminum pouch with its desiccant.
- Do not use reagents beyond the expiration date.
- Do not use reagents from other kits.
- Monitor the quality of the water used.
- The stop solution contains 1 M phosphoric acid. Handle this product with caution.
- Dispose of the equipment used in accordance with current legislation on environmental protection and management of biological waste.
- Keep the TMB solution away from light

E. Solution Preparation

- The washing solution must be diluted 20 times in distilled / demineralized water. The solution crystallizes spontaneously when cold. Bring the vial to 21 ± 3 ° C for the crystals to disappear; mix the solution carefully and collect the necessary volume.
- The dilution buffer must be diluted 5 times in distilled / demineralized water. The dilution buffer is yellow.
- The conjugate is to be diluted 50 times in the diluted dilution buffer.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless. If a blue color is visible, this would indicate a contamination of the solution or the pipette

F. Sample Preparation

The blood serum samples and the kit's references (positive and negative serum) must be diluted 100 times in the diluted dilution buffer.
Avoid using haemolysed samples or samples containing coagula.

Recommended dilution:
10µl sample + 990µl diluted dilution buffer.

G. Procedure

- All components must be brought to 21 ± 3 ° C before use.
- Read previous points carefully.

1. Distribute the diluted samples and kit's reference at the rate of **100 µl per well**. One well per sample. Cover with a lid and incubate the plate at **21 ± 3°C** for **60 ± 5 min**.

2. Dispose of the contents of the microplate. **Wash the microplate 3 times with 300 µl of washing solution**. Avoid the formation of bubbles in the wells and the drying of the microplate between each wash.

3. Add **100 µl of the diluted conjugate solution** to each well. Cover the plate with a lid and incubate at **21 ± 3°C** for **60 ± 5 min**.

4. Dispose of the contents of the microplate. **Wash the microplate 3 times with 300 µl of washing solution**. Avoid the formation of bubbles in the wells and the drying of the microplate between each wash.

5. Dispense **100 µl of TMB solution** into each well.

6. Incubate **10 ± 1 min at 21 ± 3°C** away from light, without covering.

7. Dispense **50 µl of stop solution** into each well. The color changes from blue to yellow.

8. Record optical densities with a plate spectrophotometer using a 450 nm filter within 5 minutes of adding the stop solution.

I. Result Validation

The test can be validated only if :

- the difference between the optical density readings of the positive serum and negative serum (OD positive serum - OD negative serum) at ten minutes is greater than 0,700

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

- the negative serum yields an optical density that is lower than 0,400.

$$OD_{\text{negative serum}} < 0{,}400$$

J. Result Interpretation

Calculate each serum's coefficient (S/P %) by means of the following formula:

$$S/P\ \% = \frac{OD_{\text{sample}} - OD_{\text{negative serum}}}{OD_{\text{positive serum}} - OD_{\text{negative serum}}} * 100$$

Result	Status
S/P % < 45 %	Negative
S/P % ≥ 45 %	Positive

Get a quick and easy interpretation of your results with our free online platform **AnalysiScreen** available on our website at : https://www.biox.com

ANALYSISCREEN

AnalysiScreen™ is the new application to read and interpret all ELISA plate types Monoscreen™ and Multiscreen™.

AnalysiScreen™ is :

- Available on our website : https://www.biox.com
- Updated in real time
- Compatible with all plate formats of Bio-X Diagnostics
- Very easy to use

SCAN ME

Notes*

Sample dilution 1/100
Positive and negative serum dilution 1/100

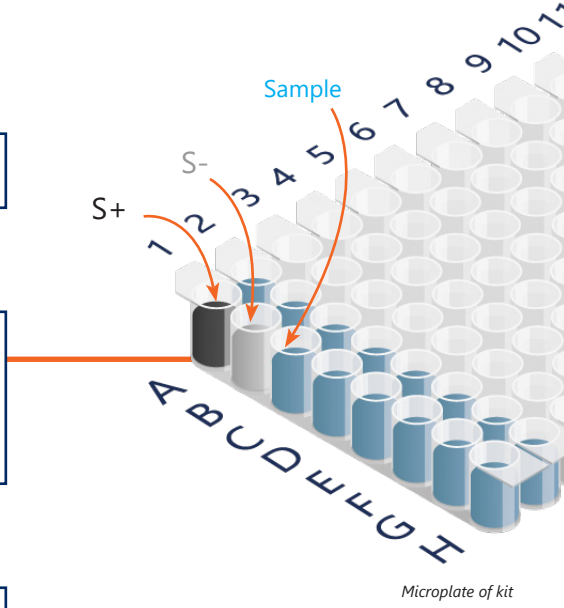
Add 100 µl of sample and references

Add 100 µl of conjugate

Add 100 µl of TMB

Add 50 µl of Stop solution

Record optical densities



Microplate of kit

* Notes are a summary of the instructions for use and cannot substitute the latter