



MONOSCREEN[®] Ab ELISA

Instruction manual
BIOK447-Ascaris suum_NO_(EN)_V02
07/03/2024

Monoscreen AbELISA *Ascaris suum*

Reference : BIO K 447

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

Monowell, indirect test

For veterinary *in vitro* use only



Sample	Pig
Blood serum	✓

Presentation

Product reference	BIO K 447/2
Format	2 plates, strips of 8 wells
Reactions	192 tests

Kit composition

Matériels fourni	BIO K 447/2
Microplates	2
Washing solution (20X)	1 x 100 mL
Colored dilution buffer (5X)	1 x 50 mL
Single component TMB Solution (1X)	1 x 25 mL
Stopping solution (1X)	1 x 15 mL
Conjugate (50X)	1 x 0,5 mL
Positive control	1 x 0,5 mL
Negative control	1 x 0,5 mL

Revision history

Date	Version	Modifications
07/03/2024	V02	Layout modification and simplification of the entire manual

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

A. Introduction

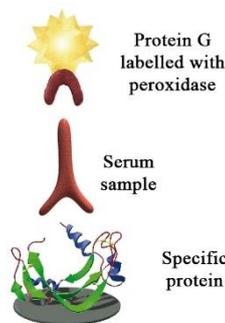
Ascaris suum is a nematode worm that infects pigs. It is spread worldwide and despite the availability of effective anthelmintics, it is still very prevalent. It can be present in groups of animals of different ages. Most of the time, ascariasis is subclinical even if in the most serious cases, respiratory 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 postmortem (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 sensitized by a specific protein from *Ascaris suum*. The entire surface of each microplate has been sensitized 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 $21 \pm 3^\circ\text{C}$. 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 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.



C. Material required but not provided

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

D. Warnings and precautions of use

- Store reagents between $+2$ and $+8^\circ\text{C}$. 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. Preparation of the solutions

- The washing solution must be diluted 20 times in distilled / demineralized water. The solution crystallizes spontaneously when cold. Bring the vial to $21 \pm 3^\circ\text{C}$ 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. Preparation of the samples

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

Recommended dilution:

10 μl sample + 990 μl diluted dilution buffer.

G. Procedure

- All components must be brought to $21 \pm 3^\circ\text{C}$ 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 **$21 \pm 3^\circ\text{C}$ for 60 \pm 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 **$21 \pm 3^\circ\text{C}$ for 60 \pm 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.
Incubate **10 \pm 1 min** at **$21 \pm 3^\circ\text{C}$** away from light, without covering.
 6. Dispense **50 μl of stopping solution** into each well. The color changes from blue to yellow.
 7. Record optical densities with a plate spectrophotometer using a **450 nm filter within 5 minutes** of adding the stopping solution.

H. Validation of results

The test can only be **validated** 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 0,450.

$$OD_{\text{positive serum}} - OD_{\text{negative serum}} > 0,450$$

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

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

I. Interpretation of results

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

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

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

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



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



Notes*

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



Add 100 µL of sample and references



Add 100 µL of diluted conjugate



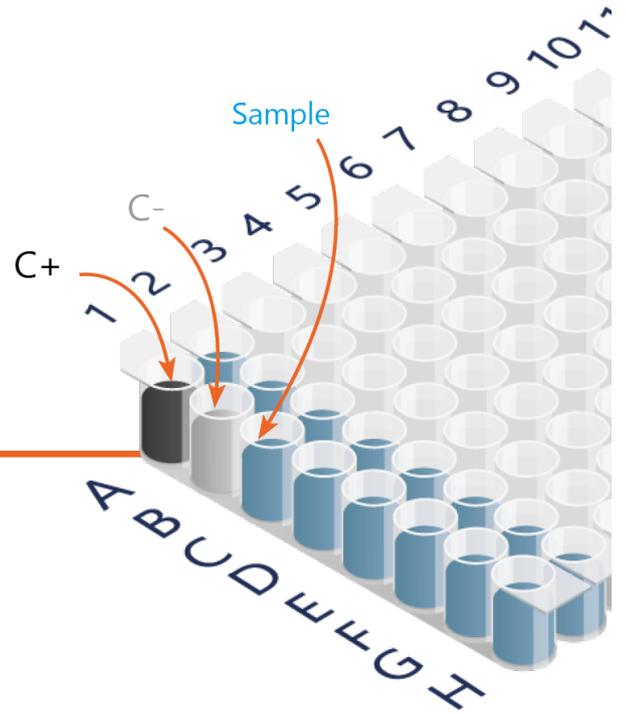
Add 100 µL of TMB



Add 50 µL of stop solution



Record optical densities



Kit microplate

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