PIGTYPE® Trichinella Ab

ELISA Test Kit for the Detection of Antibodies to *Trichinella* spp. in Pigs

Labor Diagnostik Leipzig

Instructions for Use

In vitro Diagnostic Kit for pigs
registered in accordance with § 17 c of the German Law on Animal Diseases
Registration No.: FLI-B 511

Applications

The PIGTYPE® Trichinella Ab is an indirect enzyme immunoassay (ELISA) in microtitre plate format for the detection of antibodies to *Trichinella* spp. in serum, plasma and meat juice samples from pigs.

General Information

Trichinellosis is a zoonosis which is caused by the nematode *Trichinella.* In humans the infection can cause mild to lethal illness. Humans can be infected by eating meat containing *Trichinella*-larvae. Most infected pigs do not show clinical signs. To prevent human infections, all pigs slaughtered for human consumption are tested by artificial digestion.
The European Commission Regulation (EC) No. 2075/2005 allows holdings to be certified as Trichinella-free under certain conditions. One such condition is a monitoring program. Alternatively to the cost-intensive digestion method a serological monitoring for *Trichinella*-antibodies is possible for the surveillance of pork from integrated production systems.
PIGTYPE® Trichinella Ab can be used for monitoring Trichinella infections as part of such programs.

Description of the Test Principle

The microtitre plate is coated with inactivated *Trichinella* antigen (E/S-antigen). During the sample incubation *Trichinella*-specific antibodies bind to the immobilised antigen; unbound material is removed by rinsing. The anti-IgG-HRP conjugate detects antibodies bound to the antigen. Unbound conjugate is rinsed out. The colour reaction is started by adding the substrate solution and stopped after 10 minutes. The optical density (OD) is measured in a spectrophotometer; the OD values correlate with the concentration of anti-*Trichinella* antibodies in the sample.

Ordering Information

PIGTYPE® Trichinella Ab	1 plate kit (strips)	Cat. No. 01-401/1
	5 plate kit (strips)	Cat. No. 01-401/5
	20 plate kit (solid)	Cat. No. 01-401/20

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Reagents

		1 plate kit	5 plate kit	20 plate kit
1.	Test Plate, contains 12 microtitre strips with 8 wells each	1	5	
	or Test Plate, microtitre plate with 96 wells, coated with non-infectiuos *Trichinella* E/S-antigen			20
2.	Dilution Buffer, buffer with Tween and preservative, ready-to-use	60 ml	2x 125 ml	1.0 l
3.	Negative Control, *Trichinella*-negative pig serum in buffer with protein stabilizers and preservative, ready-to-use	1.5 ml	3.5 ml	2x 3 ml
4.	Positive Control, *Trichinella*-negative pig serum in buffer with protein stabilizers and preservative, ready-to-use	1.5 ml	3.5 ml	2x 3 ml
5.	Wash Buffer (10×), buffer solution with Tween and preservative	125 ml	2x 125 ml	1.0 l
6.	anti-IgG-HRP Conjugate, anti-pig IgG-horseradish peroxidase conjugate in buffer with protein stabilisers and preservatives, ready-to-use	12 ml	60 ml	240 ml
7.	TMB (Tetramethylbenzidine) Substrate Solution, ready-to-use	12 ml	60 ml	240 ml
8.	Stop Solution, 0.5 M sulfuric acid, ready-to-use, caution!	12 ml	60 ml	240 ml

Additional Material and Equipment Required

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipetting troughs, lid, aluminium foil or adhesive for covering the Test Plate, device for delivery and aspiration of wash solution, microtitre plate spectrophotometer, tubes or plates for diluting the samples, distilled water, meat juice container (SALMOSTORE) for collecting meat juice.

Storage, Precautions and Warnings

Store the reagents at 2-8 °C and only bring them to room temperature (18-25 °C) immediately before use. Wash Solution (10x, bottle 5) and Stop Solution (bottle 8) may be stored at room temperature. Diluted Wash Solution should be stored for a maximum of 24 hours at room temperature or 1 week at 2-8 °C. Store the remaining test strips in the re-sealed foil pouch with desiccant at 2-8 °C until next use. The test strips can be stored at least for 6 weeks after opening the plate pouch.

The test should be performed by persons qualified for laboratory work. Store the TMB Substrate Solution in the dark and do not expose it to intense light or to sunlight during the performance of the test. The components of the test kit should not be contaminated or mixed with components from other batches. Do not use the components of the test kit past expiration date. Water from ion-exchange systems used for diluting the Wash Solution (10x) may interfere with the assay if not pure enough. Water of the quality of double distilled water or highly purified water (Milli-Q) is suitable.

To guarantee the precision of the results, it is absolutely essential to follow the usual precautions for ELISA procedures, including the use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times.
Stop solution contains 2.5 % sulfuric acid, caution!
All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Preparation of the Reagents

Wash Solution:
Wash Solution (10x), bottle 5, dilute 1:10 with distilled water,
e.g., for one Test Plate dilute 50 ml Wash Solution (10x) in 450 ml distilled water and mix.

Meat juice:
Freeze approx. 10 g blood- and fat-free muscle meat, e.g. from the muscular pillars of lumbar diaphragm, in a meat juice sampling device (e.g. SALMOSTORE) and then thaw. In monitoring programmes, the meat sampling sites on the carcass can be laid down exactly. Frozen meat samples can be stored for several months at -20 °C before they are assayed. The meat juice released from the thawed pork samples in the tube of the meat juice device. Thawed meat juice samples should be stored cool and analysed within 24 hours.

Dilute the meat juice samples 1:10 with Dilution Buffer before use, e.g. 25 µl sample is diluted in 225 µl Dilution Buffer and mix. Make sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.
Alternatively, meat juice samples can be diluted directly in the Test Plate (see Test Procedure, 1. Filling the Test Plate).

Serum, plasma:
Dilute the serum or plasma samples 1:100 with Dilution Buffer, e.g. 5 µl sample is diluted in 495 µl Dilution Buffer and mix. Make sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.
Alternatively, serum or plasma samples can be diluted from a pre-dilution (1:10 in Dilution Buffer) directly in the Test Plate (see Test Procedure, 1. Filling the Test Plate).

Serum or plasma samples can also be tested as pools of 10 (e.g. pool 10 µl of each of 10 samples). Dilute the pool samples 1:20 with Dilution Buffer, e.g. 10 µl sample is diluted in 190 µl Dilution Buffer and mix.

Test Procedure

Bring all reagents to room temperature (18-25 °C) before use and mix well.

1. Filling the Test Plate:

   Record the positions of the controls and samples in a test protocol, e.g. Negative Control (NC) = A1/B1; Positive Control (PC) = C1/D1; other positions of the samples.

   Pipette 100 µl of each of the ready-to-use Negative and Positive Control (in duplicates) and the 1:10 diluted meat juice samples and/or 1:100 diluted serum or plasma samples into the Test Plate wells. Alternatively, pipette 90 µl of Dilution Buffer in each sample well and add 10 µl of the undiluted meat juice sample and/or of the 1:10 pre-diluted serum or plasma sample. Mix well.
   Cover the Test Plate.

   Recommended template for PIGTYPE® Trichinella Ab ELISA:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NK	P5	P13	P21	P29	P37	P45	P53	P61	P69	P77	P85
B	NK	P6	P14	P22	P30	P38	P46	P54	P62	P70	P78	P86
C	PK	P7	P15	P23	P31	P39	P47	P55	P63	P71	P79	P87
D	PK	P8	P16	P24	P32	P40	P48	P56	P64	P72	P80	P88
E	P1	P9	P17	P25	P33	P41	P49	P57	P65	P73	P81	P89
F	P2	P10	P18	P26	P34	P42	P50	P58	P66	P74	P82	P90
G	P3	P11	P19	P27	P35	P43	P51	P59	P67	P75	P83	P91
H	P4	P12	P20	P28	P36	P44	P52	P60	P68	P76	P84	P92

2. Incubate for 60 min at room temperature or overnight at 2-8 °C and then empty the wells by aspiration or tapping.
3. Rinse each well 3x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
4. Add 100 µl ready-to-use anti-IgG-HRP Conjugate to each well.
5. Incubate for 30 min at room temperature and then empty the wells by aspiration or tapping.
6. Rinse each well 3x with 300 µl of prepared Wash Solution. Remove the buffer after each rinse.
7. Add 100 µl TMB Substrate Solution to each well.
8. Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.
9. Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order that the Substrate Solution was added.
10. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min after stopping the reaction. Measuring at a reference wavelength (620-650 nm) is optional.

Test Validation

For the assay to be valid the mean value (MV) of the measured OD values for the Positive Control must be ≥ 0.7; the mean value (MV) of the measured OD values for the Negative Control must be ≤ 0.2. In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

1. Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

2. Subtract the mean OD of NC from the OD of the sample and from the mean OD of PC.

3. The ratio sample (S) to mean PC (P) is calculated according to the following equation:

$$S/P\ \text{ratio} = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Evaluation 1h Sample Incubation

- **Serum samples with the S/P ratio < 0.3 are negative.** Specific antibodies to *Trichinella* could not be detected.

- **Serum samples with the S/P ratio ≥ 0.3 are positive.** Specific antibodies to *Trichinella* were detected.

Evaluation Overnight Sample Incubation

- **Serum samples with the S/P ratio < 0.45 are negative.** Specific antibodies to *Trichinella* could not be detected.

- **Serum samples with the S/P ratio ≥ 0.45 are positive.** Specific antibodies to *Trichinella* were detected.

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