Labor Diagnostik
Leipzig

SALMOTYPE® Pig Screen

ELISA Test Kit for Detection of Antibodies to *Salmonella* in Pigs

Instructions for Use

In vitro Diagnostic Kit for Veterinary Medicine,
registered in accordance with § 17 c of the German Law on Animal Diseases.
Registration No. BFAV-B 380

Applications

The SALMOTYPE® Pig Screen is an enzyme immunoassay (ELISA) in the microtitre plate format, to detect antibodies to *Salmonella* in samples from the pig, including meat juice, serum, and plasma. Antibodies to the O-antigens 1, 4, 5, 6, 7, and 12 are detected.

General Information

The quantitative determination by enzyme immunoassay of *Salmonella* antibodies in samples of meat juice or serum is an effective method of monitoring the infection status of populations over extended periods. The SALMOTYPE® Pig Screen ELISA permits the detection of antibodies to more than 90 % of the most frequently occurring *Salmonella* serotypes. According to the German regulation on swine *Salmonella* control by use of ELISA information about prevalence will be obtained, allowing the classification of pig populations in degrees of infection. Serological monitoring programmes are commonly performed according to the Danish Action Plan on *Salmonella* as model. Sampling plans and cut-off values for the classification of populations may vary between the national programmes. On the basis of the results on the serological classification of populations, additional measures for monitoring and treating populations will be laid down. The cut-off values for monitoring and for classification of populations will be adapted according to the initial regional situation and may be adjusted during the years of a monitoring and control programme.

Description of the Test Principle

The test system allows the quantitative determination of *Salmonella* antibodies in samples from pigs, including meat juice and serum. The microtitre plate is coated with *Salmonella* antigen. Specific antibodies to *Salmonella* form a complex with the antigen during the incubation of the sample in the coated well. Unbound material is removed by rinsing. The added antibody-enzyme conjugate binds to the antigen-bound antibodies in the sample. Unbound conjugate is rinsed out. A solution of a chromogenic enzyme substrate is added, leading to the formation of a coloured product by the antibody-bound enzyme. The colour reaction correlates with the concentration of anti-*Salmonella* antibodies in the sample.

Ordering information

SALMOTYPE® Pig Screen	1x 96 tests	Cat. No. 01-102/1
	5x 96 tests	Cat. No. 01-102/5
	20x 96 tests	Cat. No. 01-102/20

Reagents	Amount * 1er Kit	5er Kit	20er Kit
1. Test Plate, contains 12 microtitre strips with 8 wells each or Test Plate, microtitre plate with 96 wells, coated with inactivated *Salmonella* antigen	1	5	20
2. Dilution Buffer, with protein and preservative, ready-to-use	60 ml	2x 125 ml	1.0 l
3. Negative Control, ready-to-use, *Salmonella*-negative pig serum, in buffer with protein and preservative	1.5 ml	1.5 ml	2x 3.0 ml
4. Positive Control, ready-to-use, *Salmonella*-positive pig serum, in buffer with protein and preservative	1.5 ml	1.5 ml	2x 3.0 ml
5. Wash Buffer (10x), buffer solution with Tween and preservative	125 ml	2x 125 ml	1.0 l
6. Anti-IgG-HRP, ready-to-use, anti-pig-IgG horseradish peroxidase conjugate in buffer with protein stabilisers and preservative	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

* The one-plate pack size contains 12 strips each with 8 wells in a frame and desiccant in a re-sealable pack. Large packages in different configurations are available on request.

Storage and Shelf Life

The kit components are stable until the imprinted date.
Store the reagents at 2-8 °C. Wash Buffer (10x) may be stored at room temperature (18-25 °C) to avoid salt crystallisation. Also Stop Solution can be stored at room temperature. Store the remaining test strips in the re-sealed pack with desiccant at 2-8 °C until next use. The test strips can be stored at least for 6 weeks after opening the plate pack.

Necessary Instruments and Materials Not Included in the Order

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipetting troughs, microtitre plate spectrophotometer, microtitre plate shaker, tubes or predilution plates for diluting the samples, distilled water, meat juice container (SALMOSTORE) for isolating meat juice.

Precautions and Warnings

The test should only be performed by persons qualified for laboratory work. Bring the reagents to room temperature (18-25 °C) immediately before use. In case of salt crystallisation in the 10x Wash Buffer dissolve the salt crystals by mixing and careful warming. Do not expose the TMB Substrate Solution to intense light or to sunlight even during the performance of the test. The components of the test kit may not be contaminated or mixed with components from other batches. Do not use the components of the test kit past expiration date. The water used for diluting the buffer concentrate, particularly water from ion-exchange plants, may interfere with the reaction if it is 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 observe the usual precautions for ELISA procedures, including the use of carefully purified glass materials, careful pipetting and rinsing during the test, and keeping to constant times during the colour reaction. Stop Solution contains 0.5 M sulphuric acid, take care. All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Preparation of the Reagents

Only use distilled water. The Wash Buffer may also be prepared after the samples have been prepared or during the incubation of the Test Plate with the samples and control sera.

- Wash Buffer

Wash Buffer (10x), bottle 5, dilute 1:10 with distilled water,
e.g., for one Test Plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Preparation of the Samples

- Isolation of Meat Juice

Freeze approx. 10 g blood- and fat-free muscle meat, e.g. from the diaphragm column, in the SALMOSTORE meat juice container and then thaw. In the monitoring programmes, the meat sampling sites on the animal body can be laid down exactly. The frozen meat samples can be stored for several months at -20 °C before they are examined. The meat juice released from the thawed meat collects in the tube of the meat juice container.

- Meat Juice

Variant A)
Before use in the test, the meat juice samples are diluted 1:10 with Dilution Buffer, e.g. 25 µl are diluted in 225 µl Dilution Buffer and mixed.

Variant B)
Preparation of the meat juice samples is not necessary if the 1:10 dilution is performed by prefilling the Test Plate wells for meat juice with 90 µl Dilution Buffer, as described in the following test procedure.

- Serum, Plasma

Variant A)
Before use in the test, the serum or plasma samples are diluted 1:100 with Dilution Buffer, e.g. 5 µl serum or plasma are diluted in 495 µl Dilution Buffer and mixed.

Variant B)
A 1:10 predilution is performed, if a second 1:10 dilution is performed by prefilling the Test Plate wells for serum or plasma samples with 90 µl Dilution Buffer, as described in the following test procedure. The 1:10 predilution is performed, for example, by diluting 10 µl serum or plasma in 90 µl Dilution Buffer. It is recommended to use a polypropylene or polyethylene microtitre dilution plate for the predilution.

Performance of the Test

All reagents must be brought to room temperature (18-25 °C) before use.

1. Record the positions of the controls (duplicates) and samples on the Test Plate in a test protocol, e.g. Negative Control = A1/B1; Positive Control = C1/D1; other positions of the samples.
2. Filling the Test Plate - the procedure is dependent on the variant used to prepare the serum or plasma samples.

   Variant A)
   Pipette 100 µl of each of the ready-to-use Negative and Positive Control, the 1:10 diluted meat juice samples and/or the 1:100 diluted serum or plasma samples into the Test Plate wells. Cover the Test Plate.

Variant B)
Pipette 100 µl of each of the ready-to-use Negative and Positive Control into the Test Plate wells intended for them. Fill each of the other Test Plate wells with 90 µl Dilution Buffer. Then fill each of these wells with 10 µl of the undiluted meat juice samples and/or of the 1:10 prediluted serum or plasma samples. Mix the Test Plate for some seconds on a microtitre plate shaker and cover it.

3. Incubate for 60 min at room temperature and then empty the wells by suction or percussion.
4. Rinse 3x with 300 µl aliquots of prepared Wash Buffer. Remove the buffer after each rinse.
5. Add 100 µl ready-to-use anti-IgG HRP conjugate to each well.
6. Incubate for 30 min at room temperature and then empty the wells by suction or percussion.
7. Rinse 3-4x with 300 µl aliquots of prepared Wash Buffer. Remove the buffer after each rinse.
8. Add 100 µl TMB Substrate Solution to each well.
9. Incubate for 10 min at room temperature.
10. Halt the reaction by adding 100 µl of Stop Solution per well.
11. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min after stopping the reaction. Measurement at a reference wavelength (620-650 nm) is optional.

Test Validation

For the test to be valid, the quotient P/N of the mean OD of the Positive Control divided by the mean OD of the Negative Control must be greater than 4.0.

Calculation

The mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC) are calculated. The sample OD% value is calculated according to the following formula:

$$\text{Sample OD\% Value} = \frac{OD_{sample} \quad MV\ OD_{NC}}{MV\ OD_{PC} \quad MV\ OD_{NC}} \times 72.1\ \text{OD\%}$$

Evaluation

- The samples are used to evaluate the *Salmonella* status of the populations on the following basis:

  Samples with OD% value under 10 are rated as negative.
  Samples with OD% values from 10 to < 20 are rated as doubtful.
  Samples with OD% values ≥ 20 are rated as positive.

- For the evaluation of a population in accordance with the first stage of the Danish and German monitoring programme, samples with OD% values ≥ 40 are rated as positive.

- For the evaluation of samples or animals in specific monitoring programmes, cut-off values for positive samples may be laid down which are different from 40 OD%. The cut-off values laid down for these monitoring programmes must then be used to classify the populations.

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