FLOCKTYPE® *Salmonella*

ELISA Test Kit to detect Antibodies to *Salmonella Enteritidis* and *Salmonella Typhimurium* in Chicken and Turkey

**Instructions for Use**

*In-vitro* Diagnostic Kit for Veterinary Medicine,
registered in accordance with § 17c of the German Law on Animal Diseases.
Registration No. BGVV B-332

**Applications**

FLOCKTYPE® *Salmonella* is an enzyme immunoassay (ELISA) in the micro titre plate format for the detection of antibodies to *Salmonella* in serum and plasma of chicken and turkey. Antibodies to the O-antigens 1, 4, 5, 9 and 12 (for example *S. Enteritidis, S. Typhimurium*) are detected.

**General Information**

*Salmonella* infections are spread worldwide and are common to all poultry species. The main danger of *Salmonella* infections in poultry is the transmission of certain serotypes to man. Intermittent excretion of the enteritis bacteria makes the bacteriological recognition difficult. Therefore the enzyme immunoassay for the detection of antibodies against *Salmonella* is the efficient examination method. Antibody diagnostics with FLOCKTYPE® *Salmonella* is the preferred screening method in poultry flocks to detect *Salmonella* infections or humoral vaccination responses. The differentiation between antibodies respectively antibody titer following immunisation with *Salmonella* vaccine or infection with *Salmonella* field strains is not possible.

The FLOCKTYPE® *Salmonella* ELISA in combination with the FlockSoft™ software is capable of detecting the antibody titre in the chicken/turkey induced by vaccination or by natural infections and of quantitatively depicting the results.

It is important to analyse a statistically confirmed amount of animals with respect to the flock size and the expected immune status. In this test kit the anti-*Salmonella*-antibodies are detected via the O-antigen and positive results can be obtained after contact with different serotypes. Therefore it is recommended to confirm serologically positive results with bacteriological methods.

**Description of the Test Principle**

The micro titre plate is coated with *Salmonella*-LPS antigen mix. During the sample incubation, antibodies specific to *Salmonella* bind to the immobilised antigen; unbound material is removed by washing. Specific serum antibodies bound to the solid phase through the antigen are then detected with an anti-IgY-peroxidase conjugate. The reaction is visualised with the soluble chromogenic substrate TMB. After quenching the reaction, the optical density is measured in the photometer. This correlates with the activity or the anti-*Salmonella*-antibodies in the sample.
Reagents

1. Test Plate, contains 12 micro titre strips with 8 wells each or Test Plate, micro titre plate with 96 wells, coated with non-infectious *Salmonella* LPS-antigen
2. Wash Buffer (10x), buffer solution with Tween and preservative
3. Dilution Buffer, buffer with protein and preservative
4. Positive Control, *Salmonella* -reactive chicken serum in buffer with protein stabilisers and preservative, ready-to-use
5. Negative Control, *Salmonella* -negative chicken serum in buffer with protein stabilisers and preservative, ready-to-use
6. Anti-IgY-HRP, rabbit anti- IgY-horseradish peroxidase conjugate in buffer with protein stabilisers and preservatives, ready-to-use
7. TMB, Tetramethylbenzidine Substrate Solution, ready-to-use
8. Stop Solution, 0.5 M sulfuric acid, ready-to-use, corrosive!

Additional Material and Equipment Required

Beakers, measuring cylinders, analytical pipettes, multi channel pipettes, disposable pipette tips, pipetting troughs, micro titre plate spectrophotometer, tubes or plates for diluting the samples, distilled water

Precautions and Warnings

Store the reagents at 2-8 °C and only bring them 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. Wash Buffer (10x, bottle 2) and stop solution (bottle 8) may be stored at room temperature (18-25°C) to avoid salt crystallisation. 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.

The test should only be performed by persons qualified for laboratory work. Store the TMB substrate solution in the dark and do not expose this to intense light or to sunlight during the performance of the test. The components of the test kit must 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 washing during the test, and keeping to constant times during the colour reaction. The kit contains hazardous substances (sulphuric acid). All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Preparation of Reagents and Samples

Wash Buffer:

Wash Buffer concentrate (10x), bottle 2, 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.
Serum, plasma:

Before using the samples in the assay, dilute them 1:500 with Dilution Buffer, e.g. 1 µl sample is diluted in 499 µl Dilution Buffer and mixed. Be sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.

Alternatively, serum/plasma samples can be diluted from a pre-dilution (1:50 in Dilution Buffer) directly in the Test Plate (see Test Procedure, 1. Filling the Test Plate).

**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 1:500 diluted samples into the Test Plate wells. Alternatively, pipette 90 µl of Dilution Buffer in each well and add 10 µl of the 1:50 pre-diluted sample. Mix well and cover the Test Plate.

   **Template for FLOCKTYPE® Salmonella ELISA**

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</table>
   A | NC | S5 | S13 | S21 | S29 | S37 | S45 | S53 | S61 | S69 | S77 | S85 |
   B | NC | S6 | S14 | S22 | S30 | S38 | S46 | S54 | S62 | S70 | S78 | S86 |
   C | PC | S7 | S15 | S23 | S31 | S39 | S47 | S55 | S63 | S71 | S79 | S87 |
   D | PC | S8 | S16 | S24 | S32 | S40 | S48 | S56 | S64 | S72 | S80 | S88 |
   E |   | S1 | S17 | S25 | S33 | S41 | S49 | S57 | S65 | S73 | S81 | S89 |
   F |   | S2 | S10 | S18 | S26 | S34 | S42 | S50 | S58 | S66 | S74 | S82 | S90 |
   G |   | S3 | S11 | S19 | S27 | S35 | S43 | S51 | S59 | S67 | S75 | S83 | S91 |
   H |   | S4 | S12 | S20 | S28 | S36 | S44 | S52 | S60 | S68 | S76 | S84 | S92 |

2. Incubate for 30 min at room temperature and then empty the wells by aspiration or tapping.

3. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.

4. Add 100 µl ready-to-use anti-IgY-HRP 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 Buffer. 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.

9. Stop the reaction by adding 100 µl Stop Solution per well.

10. Calibrate the spectrophotometer against air as blank. 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 measured OD for the Positive Control must be ≥ 0.7; the measured OD for the Negative Control must be ≤ 0.2.

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 to mean PC is calculated according to the following equation:

\[ \frac{\text{OD}_{\text{Sample}} - \text{OD}(\text{MV})_{\text{NC}}}{\text{OD}(\text{MV})_{\text{PC}} - \text{OD}(\text{MV})_{\text{NC}}} \]

4. Endpoint titres are calculated from the S/P ratio at a 1:500 dilution using the following equation:

\[ \log_{10} \text{Titre} = 1.54 (\log_{10} S/P) + 3.77 \]

Data analysis, titre calculation, and the classification of the results can be easily performed by using the FlockSoft™ software. Negative test results are classified into titre group 0, doubtful results into titre group 1, and positive test results into titre groups 2 to 18 depending on the S/P ratio. Please refer to the FlockSoft™ manual for further information.

Evaluation

A) Field infection

- **Samples with the S/P ratio < 0.2 are diagnosed as negative.** Specific antibodies to *Salmonella Enteritidis* and *Salmonella Typhimurium* or other serotypes with O-antigens 1, 4, 5, 9 and 12 could not be detected.

- **Samples with the S/P ratio ≥ 0.2 and < 0.3 are diagnosed as doubtful.** Doubtful results should be grouped to the majority of the positive or negative test results. It is recommended to re-test doubtful results after a few weeks. Doubtful results from recently vaccinated animals may indicate a beginning increase in the formation of specific antibodies. Doubtful results from animals with repeated vaccinations may indicate an insufficient formation or a decrease of specific antibodies.

- **Samples with the S/P ratio ≥ 0.3 are diagnosed as positive.** Specific antibodies to *Salmonella Enteritidis* and *Salmonella Typhimurium* or other serotypes with O-antigens 1, 4, 5, 9 and 12 were detected.

B) Vaccination

For the assessment of the immune status, test results must be compared to animals with known vaccination or immune status. The specific immune status is high in case of a high S/P quotient. Reference values cannot be given due to different vaccines, different vaccination procedures, and other factors which influence the stock. Immunisation with life vaccines needs at least two inoculations to detect of doubtful or positive evaluated samples. We recommend to lay down the reference values for a stock after initial examinations.