August 2013

flocktype® Salmonella Ab Handbook

2 (catalog no. 275702)

5 (catalog no. 275703)*

For the detection of antibodies to *Salmonella enteritidis* and *Salmonella typhimurium*

Registered in accordance with § 17c of the German Law on Animal Diseases (BgVV-B 322)

REF 275702, 275703*

QIAGEN Leipzig GmbH, Deutscher Platz 5b, 04103 Leipzig, Germany

* Available only on request.

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
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Contents

Kit Contents

flocktype Salmonella Ab **Catalog no.** **Number of plates**	**275702** **2**	**275703*** **5**
Test Plate: microtiter plate with 96 wells, coated with non-infectious Salmonella LPS-antigen	2	5
Sample diluent, ready-to-use	1 x 125 ml	2 x 125 ml
Negative Control, ready-to-use	1 x 3.5 ml	1 x 3.5 ml
Positive Control, ready-to-use	1 x 3.5 ml	1 x 3.5 ml
Wash buffer (10x)	1 x 125 ml	2 x 125 ml
Conjugate, ready-to-use	1 x 24 ml	1 x 60 ml
TMB substrate, ready-to-use	1 x 24 ml	1 x 60 ml
Stop solution, ready-to-use	1 x 24 ml	1 x 60 ml
Handbook	1	1

* Available only on request.

Intended Use

The *flocktype* Salmonella Ab is a specific and sensitive ELISA for detecting antibodies to *Salmonella enteritidis* and *Salmonella typhimurium* in serum, plasma, and egg yolk samples from chickens and turkeys. The kit is approved by the Friedrich-Loeffler-Institut and registered in accordance with § 17c of the German Law on Animal Diseases (BgVV-B 322) for use in Germany for veterinary diagnostic procedures. For veterinary use only.

Symbols

Contains reagents for <N> plates


Legal manufacturer

Lot number


Use by date


Temperature limitations for storage

Handbook

Catalog number

Material number


Protect from light


For chicken and turkey samples

Storage

The components of the *flocktype* Salmonella Ab ELISA should be stored at 2–8°C and are stable until the expiration date stated on the label. Wash Buffer (10x) and Stop Solution may be stored at room temperature (18–25°C) to avoid salt crystallization. If test strips are provided with the kit, 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 for at least 6 weeks after opening the plate pouch.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION: The Stop Solution contains 0.5 M sulphuric acid.

All sample residues and objects which have come into contact with samples must be decontaminated or disposed as potentially infectious material.

24-hour emergency information
Chemical emergency or accident assistance is available 24 hours a day from:
CHEMTREC
USA & Canada ▪ Tel: 1-800-424-9300
Outside USA & Canada ▪ Tel: +1-703-527-3887 (collect calls accepted)

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *flocktype* Salmonella Ab is tested against predetermined specifications to ensure consistent product quality.

Introduction

The *flocktype* Salmonella Ab is a highly sensitive and specific solution for the detection of antibodies to *Salmonella* spp. Antibodies to the O-antigens 1, 4, 5, 9, and 12 (e.g., *S. enteritidis*, *S. typhimurium*) are detected. The *flocktype* Salmonella Ab is suitable for serum, plasma, and egg yolk samples from chicken and turkey.

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 Ab is the preferred screening method in poultry flocks to detect salmonella infections or humoral vaccination responses. The differentiation between antibodies present in samples as a consequence immunization with salmonella vaccine or infection with salmonella field strains is not possible.

The *flocktype* Salmonella Ab in combination with the FlockSoft™ software is capable of calculating the antibody titer in the chicken/turkey induced by vaccination or by natural infections and of quantitatively depicting the results.

It is important to analyze 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.

Principle

The microtiter test plate is coated with a salmonella-LPS antigen mix. During sample incubation salmonella-specific antibodies bind to the immobilized antigen. Unbound material is removed by rinsing. The anti-IgY-HRP conjugate detects serum antibodies bound to the antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding 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-salmonella antibodies in the sample.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipets (adjustable)
- Multichannel pipets (adjustable)
- Aluminum or adhesive foil for covering the Test Plate
- Device for delivery and aspiration of wash solution (optional)
- Microtiter plate absorbance reader
- Tubes or plates for diluting the samples
- Distilled water

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight during the performance of the test.
- 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 quality of double distilled water or highly purified water (Milli-Q) is suitable.
- The use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times is essential for precise test results.

Things to do before starting

- Bring reagents to room temperature (18–25°C) immediately before use. In case of precipitated salt crystals in the Wash Buffer (10x), dissolve by gentle swirling and warming.

Wash Buffer: Dilute Wash Buffer (10x) 1:10 in distilled water, for example, for one test plate dilute 25 ml Wash Buffer (10x) in 225 ml distilled water and mix.

Serum/plasma: Prior to sample analysis, with serum/plasma samples, dilute **1:500** in Sample Diluent (e.g., dilute 1 µl sample in 499 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Change pipet tips for each sample.

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

Egg yolk: Prior to sample analysis, with egg yolk samples, dilute **1:500** in Sample Diluent. Due to the viscosity of egg yolk it is recommended to dilute the egg yolk in two stages (see steps 1 and 4, page 15).

Bring the egg yolk to room temperature. Separate the egg yolks from the egg whites or beat the eggs without causing diffusion of the egg yolk.

- **Controls** are ready-to-use and do not require dilution.

Protocol: ELISA for serum and plasma samples

Please read "Things to do before starting", page 11.

Procedure

1. **Pipet 100 µl of each of the ready-to-use Negative Control (in duplicates) and Positive Control (in duplicates) and the 1:500 samples into the Test Plate wells.**

1a. **Alternatively, pipet 90 µl of Sample Diluent in each sample well and add 10 µl of the of the 1:50 pre-diluted sample. Mix well.**

   Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.

2. **Incubate for 30 min at room temperature (18–25°C).**
3. **Remove solution from the wells by aspiration or tapping.**
4. **Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.**
5. **Pipet 100 µl ready-to-use Conjugate to each well and incubate for 30 min at room temperature (18–25°C).**
6. **Remove solution from wells by aspiration or tapping.**
7. **Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.**
8. **Pipet 100 µl TMB Substrate Solution to each well.**
9. **Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.**

10. **Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.**
11. **Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.**
    Measuring at a reference wavelength (620–650 nm) is optional.

Protocol: ELISA for egg yolk samples

Please read "Things to do before starting", page 11.

Procedure

1. **Pipet 490 µl of Wash Buffer into a suitable microcentrifuge tube (e.g., Eppendorf® microcentrifuge tube) and add 10 µl of egg yolk.**
   It is recommended to use a positive displacement pipette for pipetting raw egg yolk.
2. **Vortex 3 x 10 sec.**
   If the egg yolk is not completely dissolved, further vortexing may be required.
3. **Pipet 100 µl of each of the ready-to-use Negative Control (in duplicates) and Positive Control (in duplicates) into the Test Plate wells.**
4. **Pipet 90 µl of Sample Diluent in each sample well and add 10 µl of the 1:50 pre-diluted sample. Mix well.**
   Record the positions of the controls and samples in a test protocol. The use of a multichannel pipet is recommended for the transfer of samples. Cover the Test Plate.
5. **Incubate for 30 min at room temperature (18–25°C).**
6. **Remove solution from the wells by aspiration or tapping.**
7. **Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.**
8. **Pipet 100 µl ready-to-use Conjugate to each well and incubate for 30 min at room temperature (18–25°C).**
9. **Remove solution from wells by aspiration or tapping.**
10. **Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.**

11. **Pipet 100 µl TMB Substrate Solution to each well.**
12. **Incubate for 10 min at room temperature in the dark. Begin timing after the first well is filled.**
13. **Stop the reaction by adding 100 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.**
14. **Measure the OD in the plate reader at 450 nm within 20 min after stopping the reaction.**

    Measuring at a reference wavelength (620–650 nm) is optional.

Data Interpretation

Validation criteria

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥0.7
- The MV of the measured OD value for the Negative Control (NC) must be ≤0.2

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

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

The ratio (S/P) of sample OD to mean OD of the Positive Control is calculated according to the following equation:

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

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

$$Log_{10}\ Titer = 1.54\ (Log_{10}\ S/P) + 3.77$$

Interpretation of the results

Field infection

Samples with the S/P ratio <0.2 are 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 doubtful.
Doubtful results should be grouped to the majority of the positive or negative results. It is recommended to retest doubtful results after a few weeks. Doubtful results from recently vaccinated animals may indicate the beginning of an 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 positive.
Specific antibodies to *Salmonella enteritidis* or *Salmonella typhimurium* or other serotypes with O-antigens 1, 4, 5, 9, and 12 could be detected.

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. Immunization with live vaccines needs at least two inoculations to detect doubtful or positive evaluated

samples. We recommend to lay down the reference values for a stock after initial examinations.

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Ordering Information

Product	Contents	Cat. no.
flocktype Salmonella Ab (2)	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	275702
flocktype Salmonella Ab (5)*	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	275703
Related products		
flocktype Mycoplasma Mg Ab (2)†	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274502
flocktype Mycoplasma Mg/Ms Ab (5)†	For 480 reactions: 5 Test Plates, Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274803

* Available only on request.
† Other kit sizes are available; see www.qiagen.com.

Product	Contents	Cat. no.
flocktype Mycoplasma Ms Ab (2)*	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274602
flocktype IBV Ab (2)*	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274302
flocktype IBDV Ab (2)*	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274202
flocktype AIV Ab (2)*	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	274012
flocktype NDV Ab (2)*	For 96 reactions: 2 Test Plates (strips), Wash Buffer, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate Solution, Stop Solution	275002

*Other kit sizes are available; see www.qiagen.com.

QIAGEN offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit www.qiagen.com/Animal-and-Veterinary-Testing for more information about the *bactotype*®, *cador*®, *cattletype*®, *flocktype*, *pigtype*®, and *virotype*®.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Quick guide

Sample dilution:
Serum/plasma/egg yolk 1:500

Step	
1. Sample	100 µl/well
2. Incubation	30 min RT
3. Wash	3 x 300 µl
4. Conjugate	100 µl/well
5. Incubation	30 min RT
6. Wash	3 x 300 µl
7. TMB	100 µl/well
8. Incubation	10 min RT
9. Stop	100 µl/well
10. Read	450 nm

Data interpretation

Negative	Doubtful	Positive
S/P ≤0.2	S/P ≥0.2 and <0.3	S/P ≥0.3

Notes

Notes

www.qiagen.com
Australia ■ techservice-au@qiagen.com
Austria ■ techservice-at@qiagen.com
Belgium ■ techservice-bnl@qiagen.com
Brazil ■ suportetecnico.brasil@qiagen.com
Canada ■ techservice-ca@qiagen.com
China ■ techservice-cn@qiagen.com
Denmark ■ techservice-nordic@qiagen.com
Finland ■ techservice-nordic@qiagen.com
France ■ techservice-fr@qiagen.com
Germany ■ techservice-de@qiagen.com
Hong Kong ■ techservice-hk@qiagen.com
India ■ techservice-india@qiagen.com
Ireland ■ techservice-uk@qiagen.com
Italy ■ techservice-it@qiagen.com
Japan ■ techservice-jp@qiagen.com
Korea (South) ■ techservice-kr@qiagen.com
Luxembourg ■ techservice-bnl@qiagen.com
Mexico ■ techservice-mx@qiagen.com
The Netherlands ■ techservice-bnl@qiagen.com
Norway ■ techservice-nordic@qiagen.com
Singapore ■ techservice-sg@qiagen.com
Sweden ■ techservice-nordic@qiagen.com
Switzerland ■ techservice-ch@qiagen.com
UK ■ techservice-uk@qiagen.com
USA ■ techservice-us@qiagen.com

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