PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit

ELISA for *in vitro* detection of antibodies against *Salmonella* in meat juice of pigs

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Salmonellosis is one of the most important zoonotic diseases, causing serious clinical signs in human beings. Pigs have been recognized as an important source for these *Salmonella* infections. Especially infected pig herds constitute a public health risk as a source of contamination of meat at slaughter. Surveillance and diagnosis of infected pig herds can be easily achieved by testing for *Salmonella* antibodies in serum or meat juice. The Applied Biosystems™ PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit originates from the Danish Veterinary Institute (Nielsen et al., 1995) and has been successfully applied in the control program for *Salmonella* in pigs in Denmark. Additionally, the ELISA is often used as the Gold Standard in the development of other *Salmonella* ELISA's [1].

The PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit can be used to detect infection in pigs caused by *Salmonella* strains belonging to the serogroup B, C1 and D (the most common serotypes isolated in Europe, Asia and America). The test is suitable for large-scale screening and for application in control programs of *Salmonella* infections in swine [1,2].

Test principle

The PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit is an indirect ELISA for the detection of *Salmonella* antibodies in swine and detects antibodies against *Salmonella* polysaccharide LPS O-antigens 1, 4, 5, 6, 7 and 12. Plates are coated with the purified LPS isolated from *S. Typhimurium* and *S. Choleraesuis*. The conjugate is rabbit anti-swine serum coupled to horse radish peroxidase.

Test samples are pre-diluted in a dummy plate and transferred to the corresponding wells of the test plate and incubated at room temperature (22±3°C). Subsequently plates are washed and the HRPO conjugate is added and incubated at 22±3°C. After the plates are washed the ready-to-use Chromogen (TMB) Substrate is dispensed to all wells of the test plate. After incubation at 22±3°C the color development is stopped and measured at 450 nm.

Kit components

5 plate kit for 450 samples. Store kit at 5±3°C until expiry date. See kit label for expiry date. The shelf life of diluted, opened or reconstituted components is noted below, where appropriate.

Component	Description
1: Test Plate	Five Test Plates
2: Conjugate (30x)	30x concentrate, dilute before use. One vial containing 2.2 mL Conjugate. Shelf life of diluted conjugate: 24 hours at 22±3°C.
3: Dilution Buffer (5x)	5x concentrate, dilute before use. One vial containing 60 mL Dilution Buffer. Shelf life of dilution buffer working solution: 24 hours at 22±3°C.
4: Washing Fluid (100x)	100x concentrate, dilute before use. One vial containing 60 mL Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.
5: Negative Control	One vial containing 0.5 mL Negative Control.
6: Validation Control	One vial containing 0.5 mL Validation Control. **Note:** Use of this control is optional.
7: Positive Control	One vial containing 0.5 mL Positive Control.
8: Chromogen (TMB) Substrate	Ready-to-use. One vial containing 60 mL of Chromogen (TMB) Substrate.
9: Stop Solution	Ready-to-use. One vial containing 60 mL Stop Solution.
Additional kit contents	Package Insert

Additional material required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Use	Description
Pre-dilution	Dummy plates to make pre-dilutions of the meat juice samples. We recommend U-bottom shaped plates (Cat. No. 267245). However, also other non-binding plates or tubes can be used.
General	Laboratory equipment according to national safety regulations.
Analysis of results	Plate reader. The reader has to have an appropriate filter set to read the plates at 450 nm.
Optional	Plate washer.

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit must be performed in laboratories suited for this purpose.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Notes

To achieve optimal results with the PrioCHECK™ Porc. Salmonella Ab 2.0 Strip Kit, the following aspects must be considered:

- **The Test Procedure protocol must be strictly followed.**
- It is recommended to use 6 times washing, if the assay is run on a robotic system.
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

Solutions to be made in advance

Dilution buffer working solution

The Dilution Buffer (Component 3) must be diluted 5 times in demineralized or distilled water. To perform a test with one plate, prepare 45 mL (add 9 mL Dilution Buffer (5x) to 36 mL demineralized or distilled water).

Stability of dilution buffer working solution: 24 hours at 22±3°C.

Conjugate dilution

Prepare dilution of the Conjugate (Component 2) in dilution buffer working solution. To perform a test with one plate, prepare 12 mL (add 0.4 mL concentrated Conjugate (30x) to 11.6 mL of dilution buffer working solution).

Note: The diluted conjugate is stable up to 24 hours.

Washing solution

The Washing Fluid (Component 4) must be diluted (100x) in demineralized water and is sufficient for a final volume of 6 liters. To perform a test with one plate prepare 500 mL (add 5 mL Washing Solution (100x) to 495 mL demineralized or distilled water).

Stability of washing solution: 1 week stored at 22±3°C.

Pre-dilution of the test samples 3x in a dummy plate

1. Dispense 50 µL of each test sample to the appropriate wells of the dummy plate (see Table 1).
   Note: Test samples can be added to one well (single test) or two adjacent wells (duplicate test), depending on your experimental requirements.
2. Dispense 100 µL of the dilution buffer working solution to each sample containing well, then mix the contents of the wells.
3. Shake the dummy plate(s) for 1 minute at 700 RPM (1/min).

Note: Mixing the sample with the dilution buffer working solution is essential for the test.

Incubation of control and test samples

1. Label each strip of the Test Plate (Component 1) with a marker pen.
2. Dispense 80 µL of the dilution buffer working solution to the sample wells of the Test Plate (see Table 2).
3. Transfer 20 µL of each pre-diluted sample from the dummy plate to the corresponding well of the Test Plate.
4. Dispense 90 µL of the dilution buffer working solution to the control wells of the Test Plate (see Table 2).
5. Dispense 10 µL of the Negative Control (Component 5) to wells A1 and B1.
6. Dispense 10 µL of the Positive Control (Component 7) to wells C1 and D1.
7. *(Optional)* Dispense 10 µL of the Validation Control (Component 6) to wells E1 and F1.
8. Shake the plate(s) for 1 minute at 700 RPM (1/min).
9. Incubate the Test Plate(s) for 30±3 minutes at room temperature (22±3°C).

Incubation with conjugate

1. Empty the Test Plate and wash the plate 3 times with 300 µL of diluted washing fluid. Tap the plate firmly after the last wash cycle.
2. Dispense 100 µL of the working solution of the conjugate to all wells.
3. Seal the Test Plate with plate sealer.
4. Incubate the Plate for 30±3 minutes at 22±3°C.

Incubation with Chromogen (TMB) Substrate

1. Empty the strip Test Plate and wash the plate 3 times with 300 µL of diluted washing fluid. Tap the plate firmly after the last wash cycle.
2. Dispense 100 µL of the Chromogen (TMB) Substrate (Component 8) to all wells.
3. Incubate the plate 15±1 minutes at 22±3°C.
4. Add 100 µL of the Stop Solution (Component 9) to all wells.
5. Mix the content of the wells of the plate.

Note: Start the addition of Stop Solution 15 minutes after the first well was filled with the Chromogen (TMB) Substrate. Add the Stop Solution in the same order and at the same pace as the Chromogen (TMB) Substrate was dispensed.

Reading of the test and calculating the results

1. Measure the optical density (**OD**) of the wells at 450 nm preferable within 15 minutes after color development has been stopped.
2. Calculate the **mean OD_{450}** value of the Positive Control (wells C1 and D1).
3. Calculate the **mean OD_{450}** value of the Negative Control (wells A1 and B1).
4. Calculate the corrected **OD_{450}** value of the Positive Control, Validation Control (if used), and all **samples** by subtracting the **mean OD_{450}** of the Negative Control (wells A1 and B1).
5. Calculate the percent positivity (**PP**) of all controls and of the **test samples** according to the formula below.

The **OD_{450}** of all **samples** is expressed as percent positivity (**PP**) of the **OD_{450}** of the Positive Control (**PC**) (wells C1 and D1) corrected with the **mean OD_{450}** of the Negative Control (**NC**) (wells A1 and B1).

$$PP = \left(\frac{OD_{450\ \text{test sample}} - \text{Mean } OD_{450\ NC}}{OD_{450\ PC} - \text{Mean } OD_{450\ NC}} \times 120 \right) - 16$$

Result interpretation

Validation criteria

1. The **mean OD_{450}** of the Negative Control (wells A1 and B1) must be <0.4.
2. The **mean OD_{450}** of the Positive Control (not corrected) should be >1.0.
3. If the Validation Control was used, the percent positivity of the Validation Control must be ≥40.

Not meeting these criteria is reason to discard the results of that specific Test Plate.

Note: If the **OD_{450}** of the Positive Control (not corrected) is below 1.000 possibly the Chromogen (TMB) Substrate is too cold. In that case warm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percent positivity

PP = <40%	Negative	*Salmonella*-specific antibodies are absent in the test sample.
PP = ≥40%	Positive	*Salmonella*-specific antibodies are present in the test sample.

In well-advanced Salmonella control programs the test can be used with a different cut-off (e.g. 20% **PP**). It remains in the responsibility of the respective authorities/users to implement such cut-offs.

Recommended plate layouts

The following plate layouts allow for efficient transfer of pre-diluted samples from the dummy plate to the Test Plate (X - Empty; S - Sample; P - Positive Control; N - Negative Control; V - Validation Control (optional)).

Table 1 Dummy plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	X	S	S	S	S	S	S	S	S	S	S	S
B	X	S	S	S	S	S	S	S	S	S	S	S
C	X	S	S	S	S	S	S	S	S	S	S	S
D	X	S	S	S	S	S	S	S	S	S	S	S
E	X or S[1]	S	S	S	S	S	S	S	S	S	S	S
F	X or S[1]	S	S	S	S	S	S	S	S	S	S	S
G	S	S	S	S	S	S	S	S	S	S	S	S
H	S	S	S	S	S	S	S	S	S	S	S	S

(1) If you are using the Validation Control, leave wells E1 and F1 empty.

Table 2 Test Plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	S	S	S	S	S	S	S	S	S	S	S
B	N	S	S	S	S	S	S	S	S	S	S	S
C	P	S	S	S	S	S	S	S	S	S	S	S
D	P	S	S	S	S	S	S	S	S	S	S	S
E	V or S	S	S	S	S	S	S	S	S	S	S	S
F	V or S	S	S	S	S	S	S	S	S	S	S	S
G	S	S	S	S	S	S	S	S	S	S	S	S
H	S	S	S	S	S	S	S	S	S	S	S	S

References

1. Nielsen B, Baggesen D, Bager F, Haugegaard J, Lind P (1995). *Veterinary Microbiology* 47:205–218.
2. Van der Heijden HMF (2001). First International Ring Trial of ELISA's for Salmonella-antibody. *Berl Münch Tierärztl Wschr* 389–392.

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Prionics Lelystad B.V. | Platinastraat 33 | 8211 AR Lelystad | The Netherlands

Revision history of Pub. No. MAN0013909 (English)

Rev.	Date	Description
B.0	29 October 2020	• Updated the protocol to make the use of the Validation Control optional. • Added a section for recommended plate layouts.
A.0	8 July 2019	New document. Converted the legacy document (MAN0013909 PrioCHECK Salmonella Ab porcine 2.0 meat juice 5 plt 7610660 v1.3_e.docx) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos. The product name was changed from PrioCHECK Salmonella Ab porcine 2.0 to PrioCHECK Porc. Salmonella Ab 2.0 Strip Kit.