PrioCHECK™ S. Dublin Ab Strip Kit

ELISA for *in vitro* detection of antibodies against *Salmonella* in milk of cattle

Catalog Number 7610640

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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

Salmonella infections in cattle can cause serious economical and welfare losses in the cattle Industry. Infection can be transmitted to humans by the consumption of infected meat or dairy products and cause severe health problems or even death. Infections caused by *Salmonella* strains belonging to serotypes B, C1 and D are the most frequent occurring and serious infectious. *Salmonella* Dublin (serotype D) is adapted to cattle and unlike most other types of *Salmonella* bacteria, has the tendency to persist in herds for decades. Additionally *Salmonella* Dublin infections in humans are extremely invasive, and when compared to other *Salmonella* infection, mortality rate is high. In order to control the infection in infected herds it is necessary to cull the carriers and prevent production of new carriers. In Europe *Salmonella* programs to control infections in swine, poultry and eggs are already implemented or in the process of being implemented. Additionally some countries already implemented control programs for *Salmonella* in cattle. The Applied Biosystems™ PrioCHECK™ S. Dublin Ab Strip Kit originates from the Danish Veterinary Institute and has been successfully applied in the control program for *Salmonella* in Denmark since 2002.

The PrioCHECK™ S. Dublin Ab Strip Kit can be used to specifically detect infections caused by *Salmonella* Dublin, however cross reaction because of the O-antigen factors 1, 9 and 12 will occur. The test is suitable for large-scale screening of serum and (bulk) milk samples.

Test principle

The PrioCHECK™ S. Dublin Ab Strip Kit is an indirect ELISA for the detection of *Salmonella* antibodies in cattle directed against *Salmonella* Dublin and detects antibodies against *Salmonella* polysaccharide LPS O-antigens 1, 9 and 12. Plates are coated with the purified LPS isolated from *Salmonella* Dublin. The conjugate is goat- anti bovine IgG coupled to horse radish peroxidase. Test samples are placed in the 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 room temperature (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 the expiry date. See kit label for actual 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 of Conjugate. Diluted Conjugate is not stable, prepare just before use.
3: Dilution Buffer (5x)	5x concentrate, dilute before use. One vial containing 60 mL of Dilution Buffer. Shelf life of dilution buffer working solution: 2 weeks at 22±3°C.
4: Washing Fluid (200x)	200x concentrate, dilute before use. One vial containing 60 mL of Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.
5: Negative Control	One vial containing 0.5 mL of Negative Control.
6: Validation Control	One vial containing 0.5 mL of Validation Control.
7: Positive Control	One vial containing 0.5 mL of 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 of Stop Solution.
Additional kit contents	• Package insert • 15 plate sealers

Additional material required

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

Use	Description
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™ S. Dublin Ab 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™ S. Dublin Ab Strip Kit, the following aspects must be considered:

- **The Test Procedure protocol must be strictly followed.**
- 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).

Shelf life of dilution buffer working solution: 2 weeks 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 to 11.6 mL of dilution buffer working solution).

Note: The diluted conjugate must be prepared just before use.

Washing solution

The Washing Fluid (Component 4) must be diluted (200x) in demineralized water and is sufficient for a final volume of 12 liters. To perform a test with one plate, prepare 500 mL (add 2.5 mL Washing Solution (200x) to 497.5 mL demineralized or distilled water).

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

Incubation of control and test milk samples

1. Label each strip of the Test Plate (Component 1) with a marker pen.
2. Dispense 100 µL of the test milk samples to wells G1–H12 of the Test Plate.
3. Dispense 90 µL of the dilution buffer working solution to the wells A1–F1 of the Test Plate.
4. Dispense 10 µL of the Negative Control (Component 5) to wells A1 and B1.
5. Dispense 10 µL of the Validation Control (Component 6) to wells C1 and D1.
6. Dispense 10 µL of the Positive Control (Component 7) to wells E1 and F1.
7. Seal the test plate(s) with a plate sealer(s).
8. Shake the plate(s) during 1 minute, level 700 (for example SLT micro plate shaker EAS 2/4, rpm 1/min level 700, SLT lab instruments).
9. Incubate the Test Plate(s) for 60±5 minutes at room temperature (22±3°C).

Incubation with Conjugate

1. Empty the Test Plate and wash the plate 6 times with 200 to 300 µL 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 a plate sealer.
4. Incubate the plate(s) for 60±5 minutes at room temperature (22±3°C).

Incubation with Chromogen (TMB) Substrate

1. Empty the Test Plate and wash the plate 6 times with 200 to 300 µL 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(s) 15 minutes at room temperature (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(s).

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, preferably within 15 minutes after color development has been stopped.
2. Calculate the mean OD_{450} value of the Negative Control (wells A1 and B1).
3. Calculate the mean OD_{450} value of the **Positive Control** (wells E1 and F1).
4. Calculate the **corrected** OD_{450} value of the **Positive Control**, Validation Control 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 **Positive Control** (PC) (wells E1 and F1) **corrected** with the mean OD_{450} of the Negative Control (NC) (wells A1 and B1).

$$PP = (\text{corrected } OD_{450\ \text{test sample}} / \text{corrected } OD_{450\ \text{Positive Control}} \times 100) - 10$$

Result interpretation

Validation criteria

1. The mean OD_{450} of the Negative Control (wells A1 and B1) must be <0.4.
2. The OD_{450} of the **Positive Control** (not corrected) should be >1.000.
3. The percent positivity of the Validation Control must be ≥30.

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 pre-warm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percent positivity

PP = <35%	Negative	*Salmonella*-specific antibodies are absent in the test sample.
PP = ≥35%	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. It remains the responsibility of the respective authorities/users to implement such cut-offs.

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

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- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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

Revision history of Pub. No. MAN0013903 (English)

Rev.	Date	Description
B	10 February 2025	A note was removed (see Incubation of control and test milk samples).
A.0	10 September 2019	• New document. Converted the legacy document (PrioCHECK *Salmonella* Ab Bovine Dublin milk 7610640 v1.2_e.doc) 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 bovine Dublin to PrioCHECK™ S. Dublin Ab Strip Kit.