PrioCHECK™ Paratuberculosis Ab 3.0 Plate Kit

ELISA for the detection of antibodies against *Mycobacterium avium ssp. paratuberculosis* in the serum, plasma, and milk of ruminants

Catalog Numbers A65415 and A65416

Pub. No. MAN0029777 Rev. D

Technology	Species	Sample matrix	Test type	Protocol
Single-well indirect ELISA–Strip plates	Bovine Ovine Caprine	Serum Heparinized plasma Milk (bovine)	Individual (serum, heparinized plasma, and milk) Bulk (milk)[1]	Short incubation Long incubation

[1] In Spain, the use of bulk bovine milk is not approved by MAPA for the diagnosis of *Mycobacterium avium ssp. paratuberculosis*.

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.

Product description

The Applied Biosystems™ PrioCHECK™ Paratuberculosis Ab 3.0 Plate Kit (Cat. No. A65415 and A65416) is an easy-to-use enzyme-linked immunosorbent assay (ELISA). This assay enables the detection of antibodies against *Mycobacterium avium ssp. paratuberculosis* in samples (serum, heparinized plasma, or milk) from ruminants. Paratuberculosis, also known as Johne's Disease, results from an infection by *Mycobacterium avium ssp. paratuberculosis* (MAP), a gram-positive bacillus. This infection primarily targets the mesenteric ganglia and small intestine, leading to a chronic intestinal infection. Clinical symptoms include diarrhea, weight loss, and edema. These symptoms become noticeable only in the advanced stages of the disease. Disease control relies on identifying infected animals and executing culling procedures. The main diagnostic tools include bacterioscopy, bacterial culture, PCR, and ELISA tests.

To prevent cross-reactions with antibodies from other mycobacteria, the samples are preincubated with *Mycobacterium phlei* proteins.

Test principle

The test is based on the principle of an indirect ELISA.

1. Samples and controls are prepared in a pre-dilution plate and incubated with *Mycobacterium phlei* (*M. phlei*) proteins. The proteins will bind to any antibodies that could interfere with the assay.
2. The pre-incubated samples and controls are then transferred to the test plate, which is coated with MAP. Anti-MAP antibodies that are present in the sample or control bind to the MAP antigen.
3. After washing, the Protein G conjugate is added, which binds to the antibodies attached to the plate.
4. Unbound conjugate is removed through washing, then a chromogenic substrate is added. The oxidation of the substrate by the conjugate results in a blue color.
5. The reaction is stopped, and the color changes to yellow. The results can be read using an ELISA plate reader.

The intensity of the yellow color in the positive samples is proportional to the amount of anti-MAP antibodies present in the sample.

Contents and storage

Table 1 PrioCHECK™ Paratuberculosis Ab 3.0 Plate Kit (Cat. No. A65415 and A65416)

Item	Description	Cat. No. A65415 (460 tests)	Cat. No. A65416 (2,760 tests)	Storage [1]
1: Test Plate	12 strips of 8 wells	5 plates	30 plates	2–8°C
2: Serum Negative Control	Light brown solution in white bottle	700 µL	2.5 mL	2–8°C
3: Serum Positive Control	Light brown solution in white bottle	700 µL	2.5 mL	2–8°C
4: Milk Negative Control	White solution in white bottle	9 mL	30 mL	2–8°C
5: Sample Diluent[2]	Yellow solution in white bottle	90 mL	500 mL	2–8°C
6: Conjugate (30X/150X)[3]	Red solution in amber bottle	2.1 mL	15 mL	2–8°C
7: Conjugate Diluent	Transparent solution in white bottle	60 mL	450 mL	2–8°C
8: Washing Fluid (40X)	Transparent solution in white bottle	60 mL	500 mL	2–8°C
9: Chromogen (TMB) Substrate	Transparent solution in amber bottle	60 mL	500 mL	2–8°C

Item	Description	Cat. No. A65415 (460 tests)	Cat. No. A65416 (2,760 tests)	Storage [1]
10: Stop Solution	Transparent solution in white bottle	60 mL	500 mL	2–8°C
Plate Sealers		10	—	Store at room temperature (18–24°C).

[1] See the label for the expiration date.

[2] Sample Diluent contains *Mycobacterium phlei* proteins.

[3] The Conjugate is included as a 30X concentrate (for use with the short incubation protocol) and a 150X concentrate (for use with the long incubation protocol). To dilute before use, see "Prepare the reagents" on page 2.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Multiskan™ FC Microplate Photometer, or equivalent[1]	51119000
Single–channel pipette (2–200 µL)	MLS
Multi–channel pipette (20–500 µL)	MLS
Pipette tips (as recommended by pipette manufacturer)	thermofisher.com/pipettetips
Pre-dilution plate	MLS
Deionized or distilled water	MLS
Solution containers	MLS
Disposable microtubes	MLS

[1] Plate reader must be capable of measurement at 450 nm.

Procedural Guidelines

- Do not combine reagents from different kit batches. Intermixing reagents from different batches can produce inaccurate data.
- Store unused test strips in an airtight pouch with desiccant at 2–8°C until the expiration date specified on the kit packaging. Do not use strips past the expiration date. Improperly stored or expired strips may yield unreliable results.
- Throughout this user guide, room temperature is defined as 18–24°C.
- The tolerance for incubation times is ±10%.

Before you begin

Prepare serum and plasma samples

Fresh, refrigerated (8 days at 5±3°C), or frozen (1 year at < –16°C) serum can be used. It is not recommended to test highly hemolyzed samples, which can result in a false positive or false negative result. An intradermal tuberculin test could interfere with the assay and cause a false positive result.

Sera and plasma samples are tested at a 1:30 dilution with Sample Diluent obtained by a 2-steps dilution (see "Incubate samples and controls" on page 2).

Prepare milk samples

Defat the milk samples.

1. Centrifuge milk samples at 1,000 × *g* for 15 minutes.

   Alternatively, keep the samples refrigerated for at least 16 hours.
2. Use a pipette to collect a test sample from below the creamy layer.

Milk samples and milk controls are tested in a 1:2 dilution with Sample Diluent obtained by a 1-step dilution (see "Incubate samples and controls" on page 2).

Prepare the reagents

- Bring the reagents to room temperature before performing the test.
- Use disposable containers to distribute the components.
- Prepare the Milk Positive Control by diluting the 3: Serum Positive Control at a 1:17 ratio with the 4: Milk Negative Control.

  Example: For one plate, add 10 µL of the 3: Serum Positive Control to 160 µL of the 4: Milk Negative Control.
- Dilute 8: Washing Fluid (40X) at a 1:40 ratio with distilled or deionized water.

  Example: For one strip, add 500 µL of the 8: Washing Fluid (40X) to 19.5 mL of water, then mix.

  The diluted washing fluid can be stored for up to 1 month at 2–8°C.

  Note: Due to the high concentration of salts, 8: Washing Fluid (40X) can contain crystals. Before dilution, shake the bottle to dissolve any crystals.
- Dilute 6: Conjugate (30X/150X) before use.
  - **For short incubation protocol**—Dilute 6: Conjugate (30X/150X) at a 1:30 ratio with the 7: Conjugate Diluent. Example: For one plate, dilute 400 µL of the 6: Conjugate (30X/150X) in 11.6 mL of the 7: Conjugate Diluent.
  - **For long incubation protocol**—Dilute 6: Conjugate (30X/150X) at a 1:150 ratio with the 7: Conjugate Diluent. Example: For one plate, dilute 80 µL of the 6: Conjugate (30X/150X) in 11.92 mL of the 7: Conjugate Diluent.

Perform the ELISA procedure

1 Incubate samples and controls

1. Dilute samples and controls with Sample Diluent in the pre-dilution plate, according to the following table.

Item	Amount	Well position[1]
Serum or plasma test		
Sample Diluent	80 µL	All wells
Serum Negative Control (NC)	10 µL	A1 and B1
Serum Positive Control (PC)	10 µL	C1 and D1
Serum or plasma test sample	10 µL	One well (single test), or two adjacent wells (duplicate test)
Milk test		
Sample Diluent	60 µL	All wells
Milk Negative Control (NC)	60 µL	A1 and B1
Milk Positive Control (PC)	60 µL	C1 and D1
Milk test sample	60 µL	One well (single test), or two adjacent wells (duplicate test)

[1] Suggested position in the pre-dilution plate.

1 *(continued)*

2. Gently shake the plate, then incubate the pre-dilution plate for 15 minutes to 24 hours at room temperature.
3. Transfer each diluted sample and control from the pre-dilution plate to the corresponding wells in the Test Plate.

Item	Amount	Well position[1]
Serum or plasma test		
Sample Diluent	70 µL	All wells
Serum sample or control dilution from the pre-dilution plate	30 µL	Corresponding well or wells in the Test Plate
Milk test		
Milk sample or control dilution from the pre-dilution plate	100 µL	Corresponding well or wells in the Test Plate

[1] Suggested position in the Test Plate.

4. Cover the Test Plate with an adhesive plate cover, then shake gently.
5. Incubate the plate.
   - **Short incubation protocol**—Incubate the plate for 45 minutes at room temperature.
   - **Long incubation protocol**—Incubate the plate overnight (16–20 hours) at 2–8°C.

2 **Add the diluted conjugate**

1. Empty the Test Plate, then wash the wells 3 times with 350 µL/well of the diluted washing fluid according to your washing method.

IMPORTANT! Do not allow the Test Plate to dry out.

   - **Manual washing—**Repeatedly fill, then empty the wells by aspiration or inversion. After the last wash cycle, tap the plate over a paper towel or absorbent surface to remove as much liquid as possible from the wells.
   - **Using an automated plate washer—**Wash the wells, following the guidelines for your instrument.
2. Add 100 µL of the diluted conjugate to the Test Plate.
3. Cover the Test Plate with a new adhesive plate cover.
4. Incubate the Test Plate for 30 minutes at room temperature.

3 **Perform the substrate reaction, then read the plate**

1. Empty the Test Plate, then wash the wells 3 times with 350 µL/well of the diluted washing fluid according to your washing method.
   - **Manual washing—**Repeatedly fill, then empty the wells by aspiration or inversion. After the last wash cycle, tap the plate over a paper towel or absorbent surface to remove as much liquid as possible from the wells.
   - **Using an automated plate washer—**Wash the wells, following the guidelines for your instrument.
2. Add 100 µL of Chromogen (TMB) Substrate to the Test Plate.
3. Incubate the Test Plate for 15 minutes at room temperature in the dark.
4. Add 100 µL of Stop Solution to the Test Plate in the same order that Chromogen (TMB) Substrate was dispensed.
5. Gently shake the Test Plate.
6. Measure the optical density (OD) of the wells using a plate reader at 450 nm within 10 minutes after color development has stopped.

Analyze the results

Calculate the results

Calculate the average OD (Optical Density) of the PC ($OD_{m\ PC}$), and that of the NC ($OD_{m\ NC}$).

For each sample, calculate the percentage positivity (PP%):

$PP\% = (OD_{Sample} - OD_{m\ NC}) / (OD_{m\ PC} - OD_{m\ NC}) \times 100$

For negative samples, PP% may result in a value < 0%.

Validation criteria

The test is validated if the following criteria are met.

Table 2 Validation criteria by sample matrix

Sample matrix	Results	
	Short incubation	Long incubation
Serum Plasma	$OD_{m\ NC} \leq 0.300$ $OD_{m\ PC} > 0.800$	$OD_{m\ NC} \leq 0.350$ $OD_{m\ PC} > 0.800$
Milk	$OD_{m\ NC} \leq 0.300$ $OD_{m\ PC} > 0.800$	

If the above criteria are not met, read the instructions again, then repeat the test. If the results are still invalid, contact technical support.

Interpretation of results

Interpret the results according to the following tables.

Table 3 Bovine sera, plasma and milk samples

Results	Interpretation
Serum and plasma	
PP% ≥ 50	Positive
PP% ≤ 40	Negative
40 < PP% < 50	Indeterminate
Milk (individual and bulk samples)	
PP% ≥ 40	Positive
PP% ≤ 30	Negative
30 < PP% < 40	Indeterminate

Table 4 Caprine sera and plasma samples

Results	Interpretation
PP% ≥ 60	Positive
PP% ≤ 50	Negative
50 < PP% < 60	Indeterminate

Table 5 Ovine sera and plasma samples

Results	Interpretation
PP% ≥ 80	Positive
PP% ≤ 70	Negative
70 < PP% < 80	Indeterminate

Locate the Certificate of Analysis

1. Go to **thermofisher.com**.
2. In the search bar, enter ***certificate lot number***, where lot number is the lot number for your kit.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029777 D

Revision	Date	Description
D	20 November 2025	A footnote about the use of bulk bovine milk in Spain was added.
C	21 May 2024	• The bulk milk sample type was added. • The amount of tests provided in the contents and storage table was updated (see "Contents and storage" on page 1). • Minor wording changes were made in accordance with regulatory authorities.
B.0	18 January 2024	The contents and storage table was updated to include the 30-plate kit format (Cat. No. A65416).
A.0	8 December 2023	New document for the PrioCHECK™ Paratuberculosis Ab 3.0 Plate Kit.

The information in this guide is subject to change without notice.

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