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cattletype® CaPV Ab Multispecies Handbook

For detection of antibodies to *Capripox-viruses* (CaPV) in cattle, sheep, and goat

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 162

REF 5 plates (cat. no. CT270603)

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INDICAL BIOSCIENCE

Contents

Kit contents

cattletype CaPV Ab Multispecies	(5)
Cat.no.	CT270603
Number of plates	5
Test Plate: microtiter plate with 12 x 8 wells, coated with non-infectious CaPV antigen	5
Sample Diluent, ready to use	1 x 60 ml
Negative Control, 1:2 dilution required	2 x 3.5 ml
Positive Control, 1:2 dilution required	2 x 3.5 ml
Wash Buffer, 10x concentrate	3 x 125 ml
Conjugate, ready to use	1 x 60 ml
TMB Substrate, ready to use	1 x 60 ml
Stop Solution, ready to use	1 x 60 ml
Handbook	1

Intended use

The cattletype CaPV Ab Multispecies is a double antigen ELISA for the detection of antibodies to *Capripoxviruses* (CaPV) in serum samples from cattle, sheep, and goat.

The kit is approved by the Friedrich-Loeffler-Institute and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 162) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

 Legal manufacturer

 Lot number

 Use by date

 Temperature limitations for storage

 Handbook

 Catalog number

 Material number

 For samples from cattle, sheep, and goat

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of cattletype CaPV Ab Multispecies is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cattletype CaPV Ab Multispecies 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. 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 from your local sales representative or by Email request to compliance@indical.com.

CAUTION: The Stop Solution contains 0.5 M sulfuric acid.

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

Introduction

The cattletype CaP Ab Multispecies is a highly sensitive and specific solution for the detection of antibodies to *Capripoxviruses* (CaPV) in cattle, sheep, and goat.

Capripoxviruses are a genus of viruses within the family *Poxviridae* and the subfamily *Chordopoxvirinae*. These viruses are among the most significant animal poxviruses due to their impact on livestock health and economic consequences. The genus includes three primary species: *Lumpy Skin Disease Virus* (LSDV), *Sheeppox Virus* (SPPV), and *Goatpox Virus* (GTPV).

LSDV primarily affects cattle, causing symptoms such as fever, enlarged lymph nodes, and characteristic nodules on the skin. The virus is transmitted mainly through insect vectors.

SPPV is specific to sheep and leads to severe skin lesions, respiratory issues, and sometimes death. The virus spreads through direct contact with infected animals or via aerosols.

GTPV affects goats, causing similar symptoms to SPPV, including skin lesions and respiratory distress. Transmission occurs through close contact with infected animals or contaminated environments.

Movement restrictions and early detection through surveillance are crucial to minimize the spread and impact of these viruses on livestock. Effective control and prevention, including vaccination, vector management, and biosecurity measures are essential to prevent outbreaks. The cattletype CaPV Ab Multispecies is a reliable tool to detect CaPV infections or to monitor humoral vaccination responses.

Principle

The cattletype CaPV Ab Multispecies is a double-antigen ELISA. The microtiter plate is coated with recombinant CaPV antigen. During sample incubation, antibodies to the CaPV protein bind to the immobilized antigen. Unbound material is removed by rinsing. Antibodies bound to the antigen are detected by horseradish peroxidase (HRP)-conjugated CaPV antigen. Unbound conjugate is removed by rinsing. A colorimetric reaction is initiated by adding Substrate Solution and stopped after 10 minutes. If antibodies to the CaPV protein are present in the sample, a blue color develops, which turns yellow after the addition of Stop Solution. The optical density (OD) is measured in a spectrophotometer at 450 nm. The OD value correlates to the concentration of the antibodies to the CaPV protein 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.

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

Important notes

General precautions

The user should always pay attention to the following:

- Do not expose the TMB Substrate Solution to intense light or to sunlight when performing the test.
- Components of the test kit should not be contaminated.
- Do not use the components of the test kit past the expiration date.
- Water from ion-exchange systems used for diluting the Wash Buffer (10x) may interfere with the assay if not pure enough. Use double-distilled water or highly purified water (Milli-Q®).
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when performing the test.

Note: For reliable results and to avoid potential non-specific reactions due to residual conjugate, make sure to fill each well completely with Wash Buffer (approximately 400 µl) during washing steps. If possible, use an ELISA washer and the overflow mode setting.

Protocol: ELISA test procedure

Important points before starting

- Please read „Important notes“ on page 10 before starting.

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.
- Dilute Wash Buffer (10x) 1:10 in distilled water. For example, for one Test Plate dilute 50 ml Wash Buffer (10x) in 450 ml distilled water and mix.
- Serum samples: Fresh, refrigerated or previously frozen serum samples may be used.
- Serum samples as well as controls can be diluted prior to analysis (preferred). Dilute serum samples 1:2 in Sample Diluent (e.g., dilute 100 µl sample in 100 µl Sample Diluent) and mix well. Use plastic tubes or uncoated microtiter plates for dilution. Use a fresh pipet tip for each sample.

Protocol: ELISA

Please read „Things to do before starting“, page 11.

Procedure

1. If using samples and controls that were diluted prior to analysis (preferred), go to step 1a. If samples and controls should be diluted in the Test Plate, go to step 1b.

1a. Pipet 100 µl each of the 1:2 diluted Negative Control (in duplicates), the 1:2 diluted Positive Control (in duplicates) and the 1:2 diluted serum samples into the wells of the Test Plate. Proceed to step 2.
Note: Record the positions of the controls and samples in a test protocol. Cover the Test Plate.

1b. Dispense 50 µl of Sample Diluent into each sample well of the Test Plate and add 50 µl undiluted Negative Control (in duplicates), Positive Control (in duplicates), serum sample. Mix well. Proceed to step 2.
Note: Record the positions of the controls and samples in a test protocol. Mix either by using a plate shaker or by repeated pipetting up and down. 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 5x with 400 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
Note: If possible, use an ELISA washer and the overflow mode setting.

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 5x with 400 µl of prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
   Note: If possible, use an ELISA washer and the overflow mode setting.
8. Pipet 100 µl TMB Substrate Solution to each well.
9. Incubate for 10 min at room temperature (18-25°C) 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.

Data interpretation

Validation criteria

The results are valid if the following criteria are met:

- The ratio between the mean OD value for the Positive Control (PC) and the Negative Control (NC) must be ≥ 4.
- The mean OD value for the Negative Control (NC) must be ≤ 0.2.

In case of invalid assays, the test should be repeated after carefully reading the instructions for use.

Calculation

Calculate the mean value (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:

$$S/P\% = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}} * 100$$

Interpretation of the results

- Samples with an S/P% ratio < 15 % are negative.
  Specific antibodies to *Capripoxviruses* (CaPV) were not detected.
- Samples with an S/P% ratio ≥ 15 % are positive.
  Specific antibodies to *Capripoxviruses* (CaPV) were detected.

INDICAL offers a comprehensive range of products to support animal pathogen testing including ELISA kits, qPCR kits and reagents, Master Mixes and nucleic acid extraction chemistry, and instrumentation for automated extraction.

Visit www.indical.com for more information about afosa, bactotype, cador, cattletype, flocktype, IndiField, IndiMag, IndiMix, IndiSpin, pigtype, SVANOVIR, and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective product handbook or user manual.

Notes

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Limited License Agreement for cattletype CaPV Ab Multispecies

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Change index

Handbook	Version	Change
HB-2665-EN-001	April 2025	Product launch

Quick guide for cattletype CaPV Ab Multispecies

Sample dilution:

Serum, Controls 1:2, mix well

Step	Protocol
1. Sample	100 µl/ well
2. Incubation	30 min RT
3. Wash	5 x 400 µl*
4. Conjugate	100 µl/ well
5. Incubation	30 min RT
6. Wash	5 x 400 µl*
7. TMB	100 µl/ well
8. Incubation	10 min RT
9. Stop	100 µl/ well
10. Read	450 nm

* If possible, use an ELISA washer and the overflow mode setting.

Data interpretation

	Negative	Positive
Serum	S/P% < 15 %	S/P ≥ 15%

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Ordering: www.indical.com/contact
Technical Support: support@indical.com
Website: www.indical.com