General Information

This diagnostic kit is designed to detect antibodies against the capripox viruses (CPV) including lumpy skin disease virus (LSDV)? goatpox (GTPV) and sheeppox (SPPV).

It can be used with individual serum or plasma of cattle, sheep, goat and any other susceptible species.

Please contact Innovative Diagnostics for use in other species.

Description and Principle

Wells are coated with CPV purified antigen.

Samples to be tested and controls are added to the microwells. Anti-CPV antibodies, if present, form an antibody-antigen complex.

Plates are washed and the conjugate, a CPV purified antigen labeled with peroxidase (HRP), is added to the microwells. It fixes to the free Fab of the bound serum anti-CPV antibodies.

After washing in order to eliminate the excess conjugate, the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- In the presence of antibodies, a blue coloration appears which becomes yellow after addition of the Stop Solution.
- In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Note: This kit does not contain infectious material.

Kit Components

Reagents*
Microplates coated with CPV purified antigen
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 19
Dilution Buffer 12
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

* *Quantities supplied are indicated on the kit label.*

1. The conjugate, controls and substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. For detailed storage conditions of opened and/or diluted components, please refer to https://www.id-vet.com/fr/support/faq.
4. Wash and stop solutions can be used for the entire IDvet product range. Substrate solutions and dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 50 µl, 100 µl, and 500 µl.
2. Disposable tips.
3. 96-well pre-dilution microplate.
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The stop solution (0,5 M acid) may be harmful if swallowed.
3. Do not expose the substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com for more detailed information.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20 X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the “IDvet Washing Guide”, available upon request.

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Testing Procedure

Allow the reagents to come to room temperature 21°C (± 5°C) before use. Homogenize all reagents by inversion or vortexing.

1. In the ELISA microplate, add:
   - 50 µl of **Dilution Buffer 19** to each microwell.
   - 50 µl of the **Negative Control** to wells A1 and B1.
   - 50 µl of the **Positive Control** to wells C1 and D1.
   - 50 µl of **each sample to be tested** to the remaining wells.
2. Cover the plate and incubate **90 ± 9 min** at 21°C (± 5°C).
3. Empty the wells. Wash each well **5** times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
4. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 12.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 ± 3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well **5** times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
8. Add 100 µl of the **Substrate Solution** to each well.
9. Cover the plate and incubate **15 ± 2 min** at 21°C (± 5°C) in the dark.
10. Add 100 µl of the **Stop Solution** to each well in the same order as in step 8 to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Positive Control O.D. (OD_{PC}) is greater than 0.350.

$$\mathbf{OD_{PC} > 0.350}$$

✓ the ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is greater than 3.

$$\mathbf{OD_{PC}/OD_{NC} > 3}$$

Interpretation

For each sample, calculate the S/P percentage (S/P%):

$$\mathbf{S/P\,\% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100}$$

Samples presenting a S/P%:

- less than 30% are considered negative.
- greater than or equal to 30% are considered positive.

Result	Status
S/P % < 30 %	NEGATIVE
S/P % ≥ 30 %	POSITIVE

Note 1: Positive results may be confirmed by other serological techniques (virus neutralization, indirect immunofluorescence).

Note 2: The IDSoft™ data analysis program is available free-of-charge. For more information, please contact support.software@innovative-diagnostics.com.

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

Certified management system

ID Screen®
Capripox Double Antigen Multi-species

Double antigen ELISA for the detection of antibodies against capripoxviruses including lumpy skin disease virus (LSDV), sheeppox virus (SPPV) and goatpox virus (GTPV) in serum or plasma from cattle, sheep, goats or other susceptible species

For *in vitro* use

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