General Information

This diagnostic kit is designed to detect antibodies directed against the nucleoprotein of the Peste des Petits Ruminants (PPR) virus.

It can be used with sheep, goat, camelid and swine serum or plasma. For use in other susceptible species, please contact Innovative Diagnostics.

Description and Principle

The wells are coated with purified recombinant PPR nucleoprotein (NP).

The samples to be tested and the controls are added to the microwells. Anti-NP antibodies, if present, form an antibody-antigen complex which masks the NP epitopes.

An anti-NP-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free NP epitopes, forming an antigen-conjugate-HRP complex.

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 sample to be tested:

- in the absence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution.
- in the presence of antibodies, no coloration appears.

The microplate is read at 450nm.

Kit Components

Reagents*
Microplates coated with PPR recombinant nucleoprotein
Anti-NP-HRP concentrated conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 13
Dilution Buffer 4
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

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

1. The conjugate, the controls and the 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 micropipettes capable of delivering volumes of 10 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

Sample Preparation

In order to avoid differences in incubation times between samples, it is possible to prepare a 96-well plate containing the test and control samples, before transferring them into an ELISA microplate using a multi-channel pipette.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature (21°C ± 5°C) 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.

Testing Procedure

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

1. Add:
   - 25 µl of **Dilution Buffer 13** to each well.
   - 25 µl of the **Positive Control** to wells A1 and B1.
   - 25 µl of the **Negative Control** to wells C1 and D1.
   - 25 µl of each sample to be tested to the remaining wells.
2. Cover the plate and incubate:
   - **For sheep, goat and swine samples: 45 min ± 4 min** at **37°C** (± 3°C).
   - **For camelid samples: 16-20 hours** at **21°C** (± 5°C).
3. Empty the wells. Wash each well 3 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 **Conjugate 10X** to 1:10 in **Dilution Buffer 4.**
5. Add 100 µl of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min ± 3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 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 min ± 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 No. 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 Negative Control O.D. (OD_{NC}) is greater than 0.7.

$$OD_{NC} > 0.700$$

✓ the mean value of the Positive Control (OD_{PC}) is less than 30 % of the OD_{NC}.

$$OD_{PC} / OD_{NC} < 0.3$$

Interpretation

For each sample, calculate the competition percentage (S/N %).

$$S/N\% = \frac{OD_{sample}}{OD_{NC}} \times 100$$

The S/N% values are interpreted as follows:

Result	Status
SHEEP, GOAT, CAMELID SAMPLES	
S/N % ≤ 50%	POSITIVE
50% < S/N % ≤ 60%	DOUBTFUL
S/N % > 60%	NEGATIVE
SWINE SAMPLES	
S/N % ≤ 30%	POSITIVE
30% < S/N % ≤ 40%	DOUBTFUL
S/N % > 40 %	NEGATIVE

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

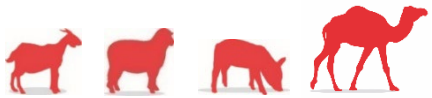
This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested)

Reference

Development of a competitive ELISA for detecting antibodies to the Peste des petits ruminants virus using a recombinant nucleoprotein. *Libeau G, Préhaud C, Lancelot R, Colas F, Guerre L, Bishop DH, Diallo A., Res Vet Sci. 1995 Jan;58(1):50-5.*

Certified management system

ID Screen®
PPR Competition



Competitive ELISA for the detection of antibodies against the PPRV nucleoprotein in serum or plasma from sheep, goat, camelid, swine or other susceptible species

For *in vitro* use

April 2022

Addition of a protocol dedicated to camelid sample testing (with an overnight sample incubation)

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