

SERELISA[®] PCV2 Ag Capture

KIT FOR THE DETECTION OF A PORCINE CIRCOVIRUS TYPE 2 (PCV2) ANTIGEN IN SWINE FAECES (INDIVIDUAL)

IMMUNOENZYMATIC TECHNIQUE

192 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] PCV2 Ag Capture kit uses a single well Sandwich immunoenzymatic technique for the detection of PCV2 antigen (Porcine CircoVirus type 2). This test is performed on faeces.

There are three steps:

1. The controls and samples are placed in wells sensitised with anti-PCV2 antibodies (Ab). Viral protein, if present in the sample, binds to the specific sites.

2. After a wash step to eliminate the non-associated fractions, an anti-PCV2/peroxidase conjugate (conj-HRP) allows the revelation of the antigen forming the following complex:

(Ab) - (Ag PCV2) - (conj-HRP)

3. After a second wash step, the enzyme coupled to the conjugate is revealed by the addition of a substrate, which transforms it into a coloured product. The optical densities are recorded and used to determine the presence or absence of the antigen as a function of threshold values.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
2 microplates containing 12 strips of 8 wells sensitised with anti-PCV2 antibodies	Use within 4 weeks after opening of the sachet which must be closed after use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Sample diluent (SD)	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	Ready-to-use.
Conjugate diluent (CD)	Ready-to-use.
Conjugate : anti-PCV2 Mab with peroxidase. (concentrated) (CJ)	To be diluted 100 or 175 times in the CD. Use within 24 hrs after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Stop solution (S)	Ready-to-use.
Adhesive films	6 films

Note: Kit and diluted reagents should be stored at + 5°C ± 3°C and used as mentioned above.

III. MATERIALS AND REAGENTS REQUIRED (NOT SUPPLIED)

- Distilled or demineralised water.
- Adjustable or set pipettes to measure and deliver between 0 to 1000 µl. *Measurement deviation must be ≤ 10% for volumes ≤ 10 µl and ≤ 5% for all other volumes.*
- Graduated cylinders (100 ml and 1000 ml).
- Manual, automatic or semi-automatic washing device for microtitration plates.
- Microplate reader, fitted with filters for bichromatic reading at 450 and 630 nm. It is also possible to use a monochromatic reader fitted with a 450 nm filter.
- Incubator at +37°C ± 3°C.
- Swabs in individual plastic tubes.

IV. PRECAUTIONS FOR USE

The quality of the results depends on the respect of good laboratory practices and the procedure (see paragraph VI).

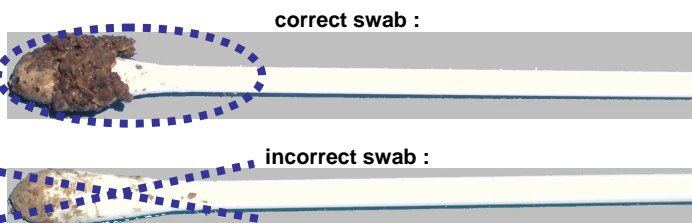
1. Do not mix or associate reagents from kits with different batch numbers
2. Do not use reagents after the expiry date.
3. Place all reagents at laboratory temperature for at least 1 hour prior to use.
4. Handle all reagents and samples as biohazardous material.
5. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
6. Never pipette by mouth.
7. Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
8. Avoid contamination of the substrate solution with metallic ions, oxidizing agents or detergents. Make sure that all containers are clean. Do not use the same container or the same pipette tip for the conjugate and the substrate.
9. It is recommended to dispose reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

Risk phrases :

- R 23/25 : Toxic by inhalation and if swallowed.
R35 : Causes severe burns.
R 36/37/38 : Irritating to eyes, respiratory system and skin.
R 41 : Risk of serious damage to eyes.
R 42/43 : May cause sensitisation by inhalation and skin contact.
S 7 : Keep container tightly closed.
S 24 : Avoid contact with skin.
S26 : In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S30 : Never add water to the product.
S45 : In case of accident or if you feel unwell, seek medical advice immediately.

V. TREATMENT AND STORAGE OF SAMPLES

The test is performed on faeces (Taking with gloves on the animal then preparation in laboratory). A swab of faeces (about 0.15 g of faeces) is diluted in 500 µl of sample diluent (SD) and centrifuged 15 minutes at 1500 g. The resulting supernatant will be tested with the dilution of the kit.



Diluted samples should be stored as follows :

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Faeces (before preparation)	max. 7 days	Yes	max. 3 days
Extracted faeces	No	Yes	No

VI. PROCEDURE

Strictly comply with the procedure indicated below. Use negative and positive controls in duplicate for each test run, for each plate.

There are 2 different protocols. Choose one and follow the procedure to the end.

	Sample incubation	Conjugate incubation
Short protocol	Time : 1 h Temperature: 37°C	Time: 1 h Temperature: 37°C Conj. dilution: 100x
Long protocol	Time: 1 night (14h-18h) Temperature: 5°C	Time: 1 h Temperature: 20°C Conj. dilution: 175x

A. PRELIMINARY STEPS

Carefully set up the distribution and identification of controls and samples.

B. TEST PROCEDURE

I - CONTROL AND SAMPLE DISTRIBUTION

1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 100 µl of negative control (N) to wells A1 and A2, and 100 µl of positive control (P) to wells B1 and B2.

2. Sample distribution:

Supernatants are tested using 100 µl per well.

Samples can be tested individually or in duplicate.

Strips should always be placed on the frame so that both washer and reader can be used.

Cover the wells with adhesive film, cut to the necessary length by the number of strips used.

Mix by gentle shaking the plate manually or by using a plate agitator.

3. Incubation of the plate:

Short protocol: 1 hour ± 5 min at + 37°C ± 3°C

Long protocol: overnight (14-18 h) at + 5°C ± 3°C.

WASHING:

Wash buffer: prepare sufficient buffer by diluting the concentrated washing solution (W) 1:10 in distilled or demineralised water.

Carefully remove the adhesive film and wash 4 times.

II – ADDITION OF CONJUGATE

1. Preparation of conjugate:

Dilute the conjugate **CJ** 1:100 or 1:175 with the diluent **CD**. 1 ml is necessary for one strip.

Dilution 100X:

10 µl of **CJ** in 990 µl of **CD**

Dilution 175X:

5.7 µl of **CJ** in 994.3 µl of **CD**

2. Distribution of conjugate:

Add 100 µl of diluted conjugate to all the wells and cover with a new piece of adhesive film.

3. Incubation of conjugate:

Short protocol: 1 hour ± 5 min. at + 37°C ± 3°C
(conjugate dilution: 1/100)

Long protocol: 1 hour ± 5 min. at + 20°C ± 5°C.
(conjugate dilution : 1/175)

WASHING :

Carefully remove the adhesive film and wash 4 times.

Reference : SCIRCO6.NA version n⁴ – 24/01/07
Version n³ → n⁴ : modification of parts III and IV

III – REVELATION

1. Addition of the substrate:

Add 100 µl of peroxidase buffered substrate (**PS**) per well.

Do not cover with adhesive film at this stage. Mix by gentle shaking the plate manually or by using a plate agitator to ensure correct mixing.

2. Incubation of substrate:

30 min. ± 5 min. at laboratory temperature (+ 20°C ± 5°C), shielded from light.

3. Addition of Stop Solution:

Add 50 µl of stop solution (**S**) per well.

Mix by gentle shaking the plate manually or by using a plate agitator. Make sure that no bubbles occur in the wells.

4. Measure of the optical density:

Measure the optical density (OD) bichromatically at 450 and 630 nm or monochromatically at 450 nm (in the yellow band).

Reading bichromatically is strongly recommended. Should a monochromatic reader be used, ensure the cleanliness of the bottom of the wells prior to reading.

VII. TEST VALIDATION

The results of each test run are valid if:

- the \overline{OD} of the negative control (N) is < 0.300, and,
- the \overline{OD} of the positive control (P) is > 0.500

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

The method for the calculation and interpretation is as follows:

METHOD BY THE CALCULATION OF THE RATIOS:

For each sample:

$$\text{Sample ratio} = \overline{OD} \text{ sample} / \overline{OD} \text{ N}$$

\overline{OD} sample = Average of the sample optical densities if the test is performed with duplicate samples.

\overline{OD} N = Average of the negative control optical densities.

Any sample presenting a ratio ≥ 2.17 is considered **positive** for the presence of PCV-2 antigen in faecal matter.

Any sample presenting $1.78 < \text{ratio} < 2.17$ is considered **doubtful** for the presence of PCV-2 antigen in faecal matter.

Any sample presenting a ratio ≤ 1.78 is considered **negative** for the presence of PCV-2 antigen in faecal matter.

Result interpretation:

	ratio 1.78	ratio 2.17	
Faeces	-	+/-	+

Should you have any question, please contact us :
SYNBIOTICS EUROPE - 2 rue Alexander Fleming
69367 LYON Cedex 07 – France
Tel : +33 4.72.76.11.11 - Fax : +33 4.72.76.11.10
www.synbiotics.fr info@synbiotics.fr

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