

## SERELISA<sup>®</sup> PCV2 Ab Mono Blocking

**KIT FOR THE DETECTION OF ANTI-PCV2 (PORCINE  
CIRCOVIRUS TYPE 2) ANTIBODIES  
IN SWINE FAECES OR SERUM  
(INDIVIDUAL)  
QUANTITATIVE METHOD ON SERUM  
QUALITATIVE METHOD ON SERUM OR FAECES**

**BLOCKING IMMUNOENZYMATIC TECHNIQUE**

384 single well reactions

### I. PRINCIPLE OF THE TEST

The SERELISA<sup>®</sup> PCV2 Ab Mono Blocking detection kit uses a single well blocking immunoenzymatic technique for the detection of anti-PCV2 (PCV2 = Porcine Circovirus type 2) antibodies in swine faeces or serum. There are three steps:

1. The controls and samples are placed in wells sensitised with anti-PCV2 antibodies (Ab1) bound specifically to purified PCV2 antigen. Anti-PCV2 antibody, if present in the faeces sample will bind with the antigen.

2. After a wash step to eliminate the non-associated fractions, an anti-PCV2/peroxidase conjugate (conj-HRP) is added. If there is no specific anti-PCV2 antibody in the sample, the anti-PCV2/peroxidase conjugate is free to attach forming the following complex:  
(Ab1) - (Ag - PCV2) - (conj-HRP)

3. After a second wash step, the coupled enzyme 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 antibodies as a function of the threshold values.

### II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 6 strips of 16 wells sensitised with anti-PCV2 antibodies bound specifically to the PCV2 antigen	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 5 days at room temperature 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 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	12 films

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

**Reference :** SCIRCO1.NA version n°5 – 31/08/2010

**The chapters modified since the last version are in italic type. Version n°4 → n°5: Quantitative method with a single dilution, test validation (VII), way of calculation and cut-off for the qualitative method on serum (VIII.(3))**

### 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).
- 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 ± 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 and safety 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. SAMPLES PREPARATION

#### V. (1) QUANTITATIVE METHOD ON SERUM USING THREE DILUTION SCHEME

The test is performed on individual serum in three wells at the following dilution: 1:100, 1:1,000 and 1:10,000 diluted in the sample diluent (SD). Each value is compared to a linear model using a logistic regression model and interpolation between the three results is correlated to a titer. (See interpretation for details about the model)

#### A. PRELIMINARY STEPS

1. Carefully set up the distribution and identification of controls and samples.
2. For the serum titration, 3 pre-dilutions of each serum are performed in hemolysis tubes or uncoated microtiter plate. Then, a 1:10 dilution of each pre-dilution is performed in the test wells.

We recommend the following scheme to prepare the appropriate set of dilutions:

Pre-dilution	Preparation
1:10	10 µl of serum + 90 µl sample diluent SD
1:100	10 µl of serum diluted 1:10 + 90 µl SD
1:1,000	10 µl of serum diluted 1:100 + 90 µl SD

## B. TEST PROCEDURE

### 1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 100 µl of negative control (N) to wells A1, A2 and A3, and 100 µl of positive control (P) to wells B1, B2 and B3.

### 2. Sample distribution:

Perform a 1:10 dilution in the test wells: dispense 90 µl of sample diluent (SD) and add 10 µl of each pre-dilution (1:10, 1:100 and 1:1,000) for each tested serum.

	1	2	3	4	5	6
A	NC	NC	NC	S7 - 1:100	S7 - 1:1000	S7 - 1:10000
B	PC	PC	PC	S8 - 1:100	S8 - 1:1000	S8 - 1:10000
C	S1 - 1:100	S1 - 1:1000	S1 - 1:10000	S9 - 1:100	S9 - 1:1000	S9 - 1:10000
D	S2 - 1:100	S2 - 1:1000	S2 - 1:10000	S10 - 1:100	S10 - 1:1000	S10 - 1:10000
E	S3 - 1:100	S3 - 1:1000	S3 - 1:10000	S11 - 1:100	S11 - 1:1000	S11 - 1:10000
F	S4 - 1:100	S4 - 1:1000	S4 - 1:10000	S12 - 1:100	S12 - 1:1000	S12 - 1:10000
G	S5 - 1:100	S5 - 1:1000	S5 - 1:10000	S13 - 1:100	S13 - 1:1000	S13 - 1:10000
H	S6 - 1:100	S6 - 1:1000	S6 - 1:10000	S14 - 1:100	S14 - 1:1000	S14 - 1:10000

**Go to step VI: PROCEDURE**

## V. (2) QUANTITATIVE METHOD ON SERUM USING A SINGLE DILUTION

The test is performed on individual serum using a single dilution: 1:1000. The optical density obtained from the reading is converted in titer according to a specific equation. (See interpretation for details about the model)

### A. PRELIMINARY STEPS

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

2. For the serum titration, a 1:10 dilution of each serum is performed in hemolysis tubes or uncoated microtiter plate. Then, a 1:10 dilution of each 1:10 pre-dilution is performed in the test wells.

We recommend the following scheme to prepare the appropriate set of dilutions:

Dilution	Preparation
1:10	10 µl of serum + 90 µl sample diluent SD
1:100	10 µl of serum diluted 1:10 + 90 µl SD

## B. TEST PROCEDURE

### 1. Control distribution:

Controls are ready-to-use.

After shaking the vials, add 100 µl of negative control (N) to wells A1, A2 and A3, and 100 µl of positive control (P) to wells B1, B2 and B3.

### 2. Sample distribution:

Perform a 1:10 dilution in the test wells: dispense 90 µl of sample diluent (SD) and add 10 µl of each 1:100 pre-dilution for each tested serum.

	1	2	3	4	5	6
A	N	N	N	S19 - 1:1000	S27 - 1:1000	S35 - 1:1000
B	P	P	P	S20 - 1:1000	S28 - 1:1000	S36 - 1:1000
C	S1 - 1:1000	S7 - 1:1000	S13 - 1:1000	S21 - 1:1000	S29 - 1:1000	S37 - 1:1000
D	S2 - 1:1000	S8 - 1:1000	S14 - 1:1000	S22 - 1:1000	S30 - 1:1000	S38 - 1:1000
E	S3 - 1:1000	S9 - 1:1000	S15 - 1:1000	S23 - 1:1000	S31 - 1:1000	S39 - 1:1000
F	S4 - 1:1000	S10 - 1:1000	S16 - 1:1000	S24 - 1:1000	S32 - 1:1000	S40 - 1:1000
G	S5 - 1:1000	S11 - 1:1000	S17 - 1:1000	S25 - 1:1000	S33 - 1:1000	S41 - 1:1000
H	S6 - 1:1000	S12 - 1:1000	S18 - 1:1000	S26 - 1:1000	S34 - 1:1000	S42 - 1:1000

**Go to step VI: PROCEDURE**

## V. (3) QUALITATIVE METHOD ON SERUM

The test is performed on individual serum diluted at 1:100 in the sample diluent (SD).

### A. PRELIMINARY STEPS

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

2. For the serum dilution, a pre-dilution 1:10 is performed in hemolysis tubes or uncoated microtiter plate. Then, a 1:10 dilution of the pre-dilution is performed in the test well.

## B. TEST PROCEDURE

### 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:

Place 100 µl of the 1:100 diluted samples per well.

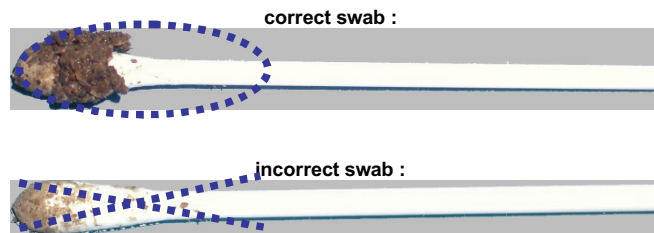
For diluting directly in the wells, place 90 µl of sample diluent plus 10 µl of a pre-diluted 1:10 serum in the well.

Samples can be tested individually or in duplicate.

**Go to step VI: PROCEDURE**

## V. (4) QUALITATIVE METHOD ON FAECES

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.



### A. PRELIMINARY STEPS

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

2. Prepare the faeces samples to be tested at a 1:10 dilution either beforehand in hemolysis tubes, uncoated microtiter plate, or directly in the test wells.

## B. TEST PROCEDURE

### 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:

Place 100 µl of the 1:10 diluted samples per well.

For diluting directly in the wells, place 90 µl of sample diluent plus 10 µl of sample in the well.

Samples can be tested individually or in duplicate.

**Go to step VI: PROCEDURE**

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (+23°C)
Serum	max. 7 days	Yes	No
Faeces (before preparation)	max. 7 days	Yes	max. 3 days
Extracted faeces	No	Yes	No

## VI. PROCEDURE

### I - CONTROL AND SAMPLE INCUBATION

- 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.

#### Incubation of the plate:

1 hour ± 5 min at + 37 ± 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 with the diluent **CD** (1 ml is necessary for one strip, meaning 10 µl of **CJ** in 990 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:

1 hour ± 5 min at + 37 ± 3°C

#### WASHING:

Carefully remove the adhesive film and wash 4 times.

### 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 ± 5 min at laboratory temperature (+23 ± 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 > 0.800, and
- the  $\overline{OD}$  of the positive control (P) is < 0.500

## VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

### VIII. (1) QUANTITATIVE METHOD ON SERUM USING THREE-DILUTION SCHEME

The method for the calculation and interpretation is based on the following model:

$$\text{Log}(Titer) = a + b \times \text{Logit}(SNc), \text{ or}$$

$$Titer = 10^{(a+b \times \text{Logit}(SNc))}$$

With

$$SNc = \frac{OD(S) - \overline{OD}(P)}{OD(N) - \overline{OD}(P)}, a = 2.5, \text{ and } b = -0.70$$

Each "Sample to Negative corrected ratio" (SNc) is calculated for each wells and noted SNc<sub>(dilution)</sub>. For each sample the following decision pathway is used:

- if SNc<sub>(1:10000)</sub> < 0.0976, then titer > 15,000 Eu (ELISA unit)
- if 0.7437 > SNc<sub>(1:10000)</sub> > 0.0976 then

$$Titer = 10 \times 10^{a+b \times \text{Logit}(SNc_{(1:10000)})}$$

- if SNc<sub>(1:10000)</sub> > 0.7437, then

- if SNc<sub>(1:1000)</sub> < 0.7437, then

$$Titer = 10^{a+b \times \text{Logit}(SNc_{(1:1000)})}$$

- if SNc<sub>(1:1000)</sub> > 0.7437, then

- if SNc<sub>(1:100)</sub> < 0.95, then

$$Titer = 0.1 \times 10^{a+b \times \text{Logit}(SNc_{(1:100)})}$$

- if SNc<sub>(1:100)</sub> > 0.95, then

$$Titer = 0$$

For your convenience, we recommend the use of a ready-to-use Excel spreadsheet that Synbiotics will provide you upon request.

### VIII. (2) QUANTITATIVE METHOD ON SERUM USING A SINGLE DILUTION

When only the 1:1000 dilution scheme is used the following calculation is performed:

- If SNc<sub>(1:1000)</sub> < 0.7437, then

$$Titer = 10^{a+b \times \text{Logit}(SNc_{(1:1000)})}$$

- If SNc<sub>(1:1000)</sub> < 0.051, then

$$Titer = +2484$$

- If SNc<sub>(1:1000)</sub> > 0.7437, then

$$Titer = <150$$

For your convenience, we recommend the use of a ready-to-use Excel spreadsheet that Synbiotics will provide you upon request.

### VIII. (3) QUALITATIVE METHOD ON SERUM

- Calculate for each sample the corrected S/N ratio
- Calculate the average of the OD if samples are tested several times

This S/N is calculated as follows:

$$S / N = \frac{OD(S)}{\overline{OD}(N)}$$

$\overline{OD}(N)$  = Average of the negative control optical densities

Any sample presenting a ratio  $\leq 0.40$  is considered **positive** for the presence of antibodies in serum.

Any sample presenting a ratio  $> 0.40$  is considered **negative** for the presence of antibodies in serum.

Ratio 0.40

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Serum	+	-
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#### VIII. (4) QUALITATIVE METHOD ON FAECES

- Calculate for each sample the corrected S/N ratio
- Calculate the average of the OD if samples are tested several times

This S/N is calculated as follows:

$$S / N = \frac{OD(S)}{\overline{OD(N)}}$$

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

Any sample presenting a ratio  $\leq 0.63$  is considered **positive** for the presence of antibodies in faecal matter.

Any sample presenting  $0.63 < \text{ratio} < 0.75$  is considered **doubtful** for the presence of antibodies in faecal matter.

Any sample presenting a ratio  $\geq 0.75$  is considered **negative** for the presence of antibodies in faecal matter.

ratio 0.63      ratio 0.75

Faeces	+	+/-	-
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Should you have any question, please contact us:  
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