

## SERELISA® PRV g1 Ab Mono Blocking

### KIT FOR THE DETECTION OF ANTI-g1 GLYCOPROTEIN ANTIBODIES OF AUJESZKY DISEASE VIRUS (PRV) IN SWINE (INDIVIDUAL)

#### BLOCKING IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

The SERELISA® PRV g1 Ab kit is intended for:

- the discrimination between infected and non-infected pigs in a population vaccinated with a g1-deleted Aujeszky vaccine (g1-);
- the identification of infected pigs in a non-vaccinated population.

#### I. PRINCIPLE OF THE TEST

The SERELISA® PRV g1 Ab Mono Blocking test kit is a blocking immunoenzymatic technique that allows the detection of anti-envelope glycoprotein (g1) antibodies in swine. There are three steps:

1. Each sample is placed in a well coated with PRV envelope glycoproteins. Anti-g1 antibodies present in the sample bind onto the viral glycoprotein antigen coated to the wells.

2. After a wash step, an anti-g1 monoclonal antibody (Mab) peroxidase conjugate is added. It fixes onto the g1 sites which remained free, forming a complex:

(Ag PRV envelope glycoprotein) - (Mab anti-g1 / peroxidase).

3. Excess conjugate is then eliminated by a second wash step. The enzyme coupled to the conjugate is revealed by addition of a substrate which is transformed into a coloured product. The optical densities are recorded and interpreted as a function of threshold obtained from the controls :

- In the absence of anti-g1 antibodies in the sample, an intense coloured reaction is seen due to the transformation of the enzyme conjugate bound to the free g1 sites fixed to the solid phase.
- In the presence of anti-g1 antibodies in the sample, less enzyme conjugate is bound to the g1 sites on the solid phase and thus the coloured reaction is diminished.

#### II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 6 strips of 2 x 8 wells sensitised with the PRV envelope glycoproteins.	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 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 (concentrated) Mab anti-PRV g1 / peroxidase (CJ)	To be diluted 10 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 ± 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 ± 3°C.

#### 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 of reagents and contaminated material according to the applicable regulations. The safety data sheets for the product are available upon request.

#### Risk and safety phrases:

R35: Causes severe burns.

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

The test is performed on decanted serum or plasma diluted at 1:2 in the sample diluent (SD).

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (+23°C)
Plasma or serum	max. 7 days	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.

#### A. PRELIMINARY STEPS

1. Carefully set up the distribution and identification of controls and samples.
2. Prepare the serum or plasma samples to be tested. Dilutions can be performed either beforehand in hemolysis tubes or in a blank microplate, or directly in the test wells.

## 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:

Place 100 µl of the two-fold diluted sera or plasma samples per well. For diluting directly in the wells, place 50 µl of sample diluent plus 50 µl of serum or plasma sample in the well, and mix thoroughly.

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:

Incubate the plate either overnight (14-18 hours) at +5 ± 3°C, or for 2 hours ± 5 min. at +37 ± 3°C.

#### WASHING:

Wash buffer : dilute 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:

Prepare the conjugate solution by diluting the concentrate (CJ) 1:10 in the conjugate diluent (CD); 2 ml are needed for one strip, meaning 200 µl of CJ in 1.8 ml 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:

Incubate for 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  $\geq 0.500$ , and,
- the competition percentage of the positive control (P) is  $\geq 80\%$ .

This percentage is calculated in the following manner:

$$\% P = \frac{\overline{OD} N - \overline{OD} P}{\overline{OD} N} \times 100$$

$\overline{OD}$  = average of the optical densities.

## VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Two methods for the calculation and interpretation are possible. The first one is recommended to compare and cumulate the results obtained from different plates during different tests.

### Method 1: CALCULATION OF THE COMPETITION PERCENTAGE (% sample)

For each sample:

$$\% S = \frac{\overline{OD} N - \overline{OD} S}{\overline{OD} N - \overline{OD} P} \times 100$$

$\overline{OD}$  = average of the optical densities, if the test is performed in duplicate.

Interpretation:

SAMPLES	NEGATIVE	DOUBTFUL	POSITIVE
Serum / plasma	%S < 40 %	40 % ≤ %S < 50 %	%S ≥ 50 %

### Method 2: ANALYSIS OF OPTICAL DENSITIES

Calculate the cut off ODs corresponding to 40%, and 50% competition and compare each sample OD to the threshold cut-offs.

$$OD_{CO\ 40} = 0.60 \overline{OD} N + 0.40 \overline{OD} P$$

$$OD_{CO\ 50} = 0.50 \overline{OD} N + 0.50 \overline{OD} P$$

Result interpretation:

	OD CO 50	OD CO 40	
Serum, plasma	+	+/-	-
			Sample OD

- Positive samples correspond to either infected pigs, or pigs vaccinated with an Aujeszky vaccine (g1+).
- Negative samples correspond to non-infected pigs, possibly vaccinated with a deleted Aujeszky vaccine (g1-).
- Doubtful samples should be retested. If the doubtful result persists, a second test on a new serum or plasma sample from the same animal is recommended.

Should you have any question, please contact us:  
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