

SERELISA[®] BVD p80 Ab Mono Blocking

KIT FOR THE DETECTION OF BVD/BD VIRUS ANTIBODIES IN RUMINANTS INDIVIDUAL (BOVINE AND GOAT SAMPLES) INDIVIDUAL AND POOLS (OVINS SAMPLES)

BLOCKING IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] BVD p80 Ab Mono Blocking detection kit uses a single well blocking immunoenzymatic technique for the detection of specific antibodies to a protein common to all strains of BVD/MD and BD virus (p80/125 non-structural protein). These antibodies are introduced following virus multiplication after a natural infection or vaccination with a live virus. There are three steps:

1. Each serum or plasma sample is placed in a well sensitised with the BVD/BD p80/125 protein. Anti-BVD/BD p80/125 antibodies present in the sample bind specifically onto the antigen coated to the wells.

2. Following a wash step, an anti-BVD/BD p80/125 monoclonal antibody peroxidase conjugate is added. It fixes onto the free antigenic sites forming a complex:
(Ag) - (anti-BVD/BD p80/125 / peroxidase).

3. Excess conjugate is eliminated by a second wash step. The enzyme linked to the complex is revealed by the addition of a substrate which transforms it into a coloured product. The corresponding optical densities are read and interpreted in the following manner:

- In the absence of antibodies in the sample, an intense coloured reaction is observed due to the transformation of the enzyme conjugate bound to the free BVD/BD p80/125 sites fixed on the solid support.

- In the presence of anti-BVD/BD p80/125 antibodies in the sample, less enzyme conjugate is bound to the antigenic sites on the solid support 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 BVD/BD [p80/125] protein.	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 (concentrated) Mab anti-BVD/BD [p80/125] / peroxidase (CJ)	To be diluted 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.

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.

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 sensitization 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

The test is performed on decanted serum or plasma diluted either 1:10 for bovine sample or 1:5 for goat and ovine samples.

Ovine samples can be tested using pool of up to 5 individual samples and tested diluted 1:5.

Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Diluted 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

- Carefully set up the distribution and identification of controls and samples.
- Prepare the serum or plasma samples to be tested. Dilutions can be performed either beforehand in hemolysis tubes, 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 ten-fold diluted bovine samples per well. Place 100 µl of the five-fold diluted ovine or caprine samples per well.
- For diluting directly in the wells, place 90 µl of sample diluent plus 10 µl of bovine serum in the well, and 80 µl of sample diluent plus 20 µl of ovine or caprine 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 overnight (14-18 hours) at + 5°C ± 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 either 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 +20°C ± 5°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 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 ≥ 0.500 , and,
- the competition percentage of the positive control (P) is $\geq 70\%$.

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:

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.
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BOVINE

Any bovine serum or plasma sample presenting a competition percentage (% S) ≥ 50 % is considered positive.
Any bovine serum or plasma sample presenting a competition percentage (% S) < 30 % is considered negative.

OVINE / GOAT

Any ovine individual serum or plasma sample presenting a competition percentage (% S) ≥ 40 % is considered positive.
Any ovine individual serum or plasma sample presenting a competition percentage (% S) < 20 % is considered negative.

OVINE (pooled samples)

Any pool of samples presenting a competition percentage (%S) < 40 % is considered negative and ≥ 40 % is considered positive.

Doubtful zone :

Any serum or plasma individual sample presenting a competition percentage situated in a DOUBTFUL ZONE between 30 and 50 % for bovine sample and 20 and 40 % for ovine or goat sample should be considered doubtful and to renew the test with the same sample is advised. If the doubtful result is confirmed, a second test on a different sample from the same animal is recommended.

Notes:

- The competition percentage for samples providing an "over" or higher than 2.5 OD, is considered to be equal to 0%.
- Some particular problems can occur with sera from animals less than 6 months old, descending from animals possessing anti-BVD/BD antibodies. Colostral antibody residues may lead to doubtful or weak positive results.
- If the kit is used to identify persistently infected animals in a pestivirus-infected population older than 6 months, any sample presenting a doubtful or negative result must be considered suspect for IPI status.
- This test targets anti-p80/125 antibodies. This kit is not intended to check the efficiency of vaccination with inactivated BVD/BD vaccines.

Method 2 : ANALYSIS OF OPTICAL DENSITIES

BOVINE

Calculate the cut off ODs corresponding to 30%, and 50% competition and compare each sample OD to the cut off OD CO 30 and OD CO 50.

$$OD\ CO\ 30 = 0.70 \overline{OD} N + 0.30 \overline{OD} P$$

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

OVINE

Calculate the cut off ODs corresponding to 20%, and 40% competition and compare each sample OD to the cut off OD CO 20 and OD CO 40 for individual samples; compare each sample OD to the cut off OD CO 40 for pool of 3 samples.

$$OD\ CO\ 20 = 0.80 \overline{OD} N + 0.20 \overline{OD} P$$

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

Result interpretation:

BOVINE

	OD CO 50	OD CO 30	
Individual sample	+	+/-	-
Sample OD			

OVINE

	OD CO 40	OD CO 20	
Individual sample	+	+/-	-
Pooled sample	+	-	
Sample OD			

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