

SERELISA® BHV-1 gB Ab Mono Blocking

**KIT FOR THE DETECTION OF ANTI-gB ANTIBODIES TO
THE VIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS
(IBR) AND INFECTIOUS PUSTULAR VULVOVAGINITIS (IPV)
IN BOVINE SERUM AND PLASMA (INDIVIDUAL)
AND MILK (INDIVIDUAL)**

BLOCKING IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA® BHV-1 gB Ab Mono Blocking kit uses a single well blocking immunoenzymatic technique for the detection of antibodies anti-envelope glycoprotein gB of Bovine Herpes Virus type 1 (BHV-1) in individual bovine sera and plasma and individual milk.

There are three steps:

1. Each sample is placed in a well sensitised with the gB glycoprotein of BHV-1. Anti-gB antibodies present in the sample bind specifically onto the antigen coated to the wells.

2. Following a wash step, a conjugate combining two monoclonal antibodies (MAbs) anti-gB/peroxidase is added. It fixes onto the free antigenic sites, forming a complex :
(Ag-gB) - (MAbs anti-gB/peroxidase).

3. Excess of free 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 gB glycoprotein sites fixed to the solid support.

- In the presence of anti-gB 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 gB glycoproteins of BHV-1.	Use within 2 months after opening of the sachet which must be closed after use.
Wash solution (W) (10 times concentrated)	Dilute 10 times in distilled or demineralised water. Use within 5 days at room temperature after dilution.
Sample diluent pink color (SD)	Ready-to-use.
Negative control (N). May contain harmless precipitate.	Ready-to-use.
Positive control (P)	Ready-to-use.
Conjugate diluent blue color (CD)	Ready-to-use.
Conjugate (100 times concentrated) MAbs anti-gB/peroxidase (CJ)	To be diluted 100 times in the CD. Use within 3 hours 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. **Caution:** only the reagents to be used in the following step are concerned.
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:

R35: Causes severe burns.

R36: Irritating to eyes.

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.

S60: This material and/or its container must be disposed of as hazardous waste.

V. SAMPLES

The test is performed on individual sera or plasma and individual milk. Milk samples should be skimmed before testing (either overnight decantation or low-speed centrifugation).

Samples should be stored as follows:

Samples	Cold (+5°C)	Freeze (-20°C)	Lab Temperature (+23°C)
Serum or plasma (individual)	max. 7 days	Yes	No
Milk	max. 5 days	Yes	No

VI. PROCEDURE

Strictly comply with the procedure indicated next page. 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 and milk samples to be tested; 1:2 dilutions should be performed either before in hemolysis tubes, in a blank microplate or directly in the test wells.

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 samples per well.
- For diluting directly in the wells, place 50 µl of sample diluent (SD) plus 50 µl of sample in the well.
- Samples may 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 incubation protocol: 2 hours ± 5 min at +37 ± 3°C,
- Long incubation protocol: overnight (14-18 hours) at +5 ± 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:

Prepare the conjugate solution by diluting the concentrate (CJ) 1:100 in the conjugate diluent (CD); 2 ml are needed for one strip, meaning 20 µl of CJ in 1.980 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 30 ± 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:

15 ± 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 average optical density (OD) value of the Negative controls (A1, A2) is > 0.5.
- The P/N of the average optical density (OD) value of the Positive controls (B1, B2) is less than 0.3.

This P/N is calculated as follows:

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

OD = average of the optical densities of the negative and positive control.

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

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

This S/N is calculated as follows:

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

RESULTS

	S/N	0.5	0.55	
Individual Serum or Plasma or Milk	+	+/-	-	Values S/N

- Any **individual serum, plasma or milk** sample presenting a ratio S/N ≤ 0.5 is considered as **positive**.
- Any **individual serum, plasma or milk** sample presenting a ratio S/N > 0.55 is considered as **negative**.

Doubtful Zone:

Any **individual serum, plasma or milk** sample which presents a ratio S/N situated in a zone from 0.5 to 0.55 is considered as doubtful and should be retested. If the doubtful result is confirmed, a second test on a different sample from the same animal is recommended.

Should you have any question, please contact us:
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