

SERELISA[®] BHV-1 Total Ab Mono Indirect

**KIT FOR THE DETECTION OF ANTIBODIES TO THE VIRUS OF
INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) AND
INFECTIOUS PUSTULAR VULVOVAGINITIS (IPV)
IN BOVINE SERUM, PLASMA (INDIVIDUAL AND POOL UP TO 10)
AND IN MILK (INDIVIDUAL AND POOL UP TO 200)**

INDIRECT IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] BHV-1 Total Ab Mono Indirect kit uses a single well indirect immunoenzymatic technique for the detection of antibodies directed against the virus BHV-1 in bovine serum, plasma (individual and pool up to 10) and milk (individual and pool up to 200).

There are three steps:

- Each sample is placed in a well sensitised with the viral antigen. Antibodies present in the sample bind specifically onto the antigen coated to the wells.
- Following a wash step, an anti-bovine IgG monoclonal antibody (MAb) peroxidase conjugate is added. It fixes onto the antibodies present, forming a complex
(Ag) - (Ab) - (MAb anti-bovine IgG / peroxidase).
- 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 by using a threshold value obtained through the use of the positive control.

II. KIT COMPOSITION AND CONSERVATION

REAGENT NATURE	RECONSTITUTION AND CONSERVATION
4 microplates containing 6 strips of 2 x 8 wells sensitised with viral Ag	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 after dilution.
Sample diluent pink color (SD)	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	Ready-to-use.
Conjugate diluent blue color (CD)	Ready-to-use.
Conjugate (concentrated) anti-bovine IgG / peroxidase (CJ)	To be diluted 20 times for short incubation protocol and 25 times for long incubation protocol in the CD. Use within 2 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).

- Do not mix or associate reagents from kits with different batch numbers
- Do not use reagents after the expiry date.
- 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
- Handle all reagents and samples as biohazardous material.
- Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
- Never pipette by mouth.
- Avoid inter sample contamination during sample collection, storage or transport. Use separate disposable pipette tips for each sample.
- 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.
- 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 bovine sera, plasma (individual and pool to 10) and milk (individual or pool up to 200). Complete pool of sera or plasma will be fitted to 10 with negative sample or sample diluent (SD).

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)
Sera, plasma individual and pool to 10	max. 7 days	Yes	No
Milk individual or pool up to 200	max. 5 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, plasma and milk samples to be tested. For serum and plasma, dilutions are performed 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:

- 200 µl of negative control (N) to wells A1 and A2,
- 200 µl of positive control (P) to wells B1 and B2.

2. Sample distribution:

- Sample preparation:

- Dilute individual serum or plasma 1:20 in sample diluent directly in the well. Place 190 µl of sample diluent (SD) plus 10 µl of sample in the well.
- Dilute pool of sera or plasma 1:2 in sample diluent directly in the well. Place 100 µl of sample diluent (SD) plus 100 µl of sample in the well.
- Test the milk samples (pool up to 200 or individual) without any dilution. Place 200 µl of sample in the well.

- Samples may either 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 if possible, cut to the necessary length by the number of strips used.

3. Incubation of the plate:

- Short incubation protocol: 1 hour ± 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. In the case of clotted milk or inefficient skimming, it is recommended to increase, if necessary, the number of wash cycles after incubation of the milk samples.

Carefully remove the adhesive film and wash 4 times.

II - ADDITION OF CONJUGATE

1. Preparation of conjugate:

Prepare the conjugate solution by diluting the concentrated conjugate (CJ) in the conjugate diluent (CD); 2 ml are needed for one strip.

- 1:20 (short incubation protocol)
For example, 100 µl of CJ in 1900 µl of CD.
- 1:25 (long incubation protocol)
For example, 80 µl of CJ in 1920 µl of CD.

Use the diluted solution within 2 hours.

2. Distribution of conjugate:

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

3. Incubation of conjugate:

Incubate 30 ± 2 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 ± 2 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 OD of the positive control (P) is ≥ 0.6 , and,
- the \overline{OD} of the negative control (N) is < 0.4 .

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

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

This S/P is calculated as follows:

$$S/P = \frac{OD_{Sample} - \overline{OD}_N}{\overline{OD}_P - \overline{OD}_N}$$

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

RESULTS

Any **individual** serum/plasma sample presenting an S/P ratio ≥ 0.65 for short incubation protocol and an S/P ratio ≥ 0.35 for long incubation protocol is considered **positive**.

Any **individual** serum/plasma sample presenting an S/P ratio < 0.65 for short incubation protocol and an S/P ratio < 0.35 for long incubation protocol is considered **negative**.

Any **pool** of sera/plasma sample presenting an S/P ratio ≥ 1 for short incubation protocol and an S/P ratio ≥ 0.8 for long incubation protocol is considered **positive**.

Any **pool** of sera/plasma sample presenting an S/P ratio < 1 for short incubation protocol and an S/P ratio < 0.8 for long incubation protocol is considered **negative**.

Any **milk** sample individual or pool up to 200, presenting an S/P ratio ≥ 0.25 is considered **positive**.

Any **milk** sample presenting an S/P ratio < 0.25 is considered **negative**.

INDIVIDUAL SERUM AND PLASMA

S/P 0.65

Serum, plasma individual Short Incubation Protocol	-	+
	Values S/P	

S/P 0.35

Serum, plasma individual Long Incubation Protocol	-	+
	Values S/P	

POOL OF SERA AND PLASMA

S/P 1

Serum, plasma individual Short Incubation Protocol	-	+
	Values S/P	

S/P 0.8

Serum, plasma individual Long Incubation Protocol	-	+
	Values S/P	

MILK

S/P 0.25

Milk individual and pool up to 200	-	+
	Values S/P	

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