

## SERELISA® BLV Ab Bi Indirect

### KIT FOR THE DETECTION OF ANTI-gp 51 BOVINE LEUKOSIS VIRUS ANTIBODIES (BLV) IN BOVINE SERUM (INDIVIDUAL AND POOLS)

#### INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 double well reactions

#### I. PRINCIPLE OF THE TEST

The SERELISA® BLV Ab Bi Indirect kit uses a double well indirect immunoenzymatic technique for the detection of anti-bovine leukosis virus (BLV) envelope glycoprotein (gp 51) antibodies in serum. The reaction is composed of three steps:

- Each serum sample to be tested is placed into two adjacent wells sensitized with a cellular antigen (odd columns) and a viral antigen (even column). Antibodies in the sample fix to the antigens coated on the viral antigen well.
- 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 gp 51) - (Ab anti-gp 51) - (Mab anti-bovine IgG / peroxidase).
- Excess 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. After stopping the enzymatic reaction, the difference of the optical densities between the two adjacent wells is measured. The presence or absence of antibodies is determined by using threshold values obtained from 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 cellular Ag (odd column) or viral Ag (even column).	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate (CJ) (concentrated 100X) Mab anti-bovine IgG/peroxidase	Dilute 100 times in the conjugate diluent and use within 24 hrs after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N)	Ready-to-use.
Positive control (P)	Ready-to-use.
Sample diluent (SD)	Ready-to-use.
Wash solution (W) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Conjugate diluent (CD)	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).

- 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.
- 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 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 can be performed on individual or pooled sera (up to 10) diluted at 1:10. Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Individual or pooled sera	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 sera and pools (up to 10 sera) to be tested. 1:10 dilutions can be performed either beforehand in hemolysis tubes, 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 through A4 (A1 & A3: cellular antigen ; A2 & A4 : viral antigen). Add 100 µl of positive control (P) to wells B1 through B4 (B1 & B3: cellular antigen ; B2 & B4 : viral antigen).

#### 2. Sample distribution:

Distribute 100 µl of the 10 fold diluted samples in two adjacent wells (cellular and viral antigens). For direct in-well dilution, dispense 90 µl of sample diluent plus 10 µl 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 1 hour ± 5 min. at + 37°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) 1:100 in the conjugate diluent (CD). (2 ml are needed for one strip, meaning 20 µl of CJ diluted in 1.98 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°C ± 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:

Incubate for 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 differences in the optical densities obtained with the positive control are  $\geq 0.200$ , and

- the  $\overline{\Delta OD}$  for the N is  $< 0.40 \times \overline{\Delta OD P}$ .

## VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

The presence of anti-gp 51 bovine leukosis virus antibodies is determined by the difference in the optical densities ( $\Delta OD$ ) between the viral Ag well and the cellular Ag well. This difference is then compared to threshold values obtained from the positive control.

Two methods for the calculation and interpretation are possible:

### Method 1 : INDEX CALCULATION

Calculate the threshold value indexes:

For pooled sera: threshold value:  $- 0.0625 \times \overline{\Delta OD P}$

For individual serum: - positive threshold value: 0

- negative threshold value :  $-0.0625 \times (\overline{\Delta OD P})$

Calculate the index for each sample tested as follows:

Sample index =  $0.25 \times (\overline{\Delta OD Sample} - \overline{\Delta OD P})$

( $\overline{\Delta OD P}$ : the average of the differences of the optical densities observed for the positive control tested in duplicate).

### Pooled sera:

Any pool sample presenting an index  $\geq -0.0625 \times \overline{\Delta OD P}$  is considered as positive.

Any pool sample presenting an index  $< -0.0625 \times \overline{\Delta OD P}$  is considered as negative.

### Individual sera:

Any serum sample presenting an index  $\geq 0$  is considered as positive.

Any serum sample presenting an index  $< -0.0625 \times \overline{\Delta OD P}$  is considered as negative.

Any serum sample presenting an index between  $-0.0625 \times \overline{\Delta OD P}$  and 0 is considered as doubtful.

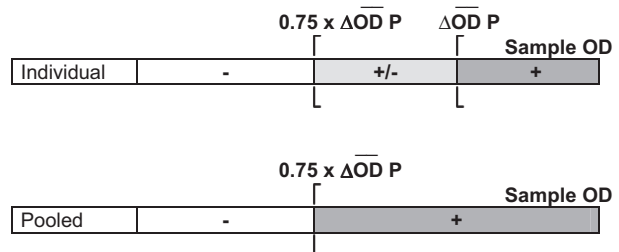
### Method 2 : ANALYSIS OF THE OPTICAL DENSITIES

Calculate the threshold values in  $\Delta OD$  corresponding to :

the  $0.75 \times (\overline{\Delta OD P})$  [threshold value for pooled and negative threshold for individual sera] and the  $(\overline{\Delta OD P})$  [positive threshold value for individual sera].

Compare each sample  $\Delta OD$ s to the thresholds [ $0.75 \times (\overline{\Delta OD P})$ ] and  $(\overline{\Delta OD P})$

### Results interpretation :



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