

SERELISA[®] BLV Ab Mono Blocking

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

BLOCKING IMMUNOASSAY TECHNIQUE

384 single well reactions

I. PRINCIPLE OF THE TEST

The SERELISA[®] BLV Ab Mono Blocking kit uses a blocking immunoassay technique permitting the detection of anti-BLV envelope glycoprotein (gp 51) antibodies in serum. The kit allows the minimum detection of one positively-infected bovine leukosis serum sample in a pool of 10 sera, according to European Union regulations. There are three steps:

- Each serum sample is placed in a well coated with the BLV gp 51 envelope glycoprotein. Anti-gp 51 antibodies present in the sample bind onto the viral glycoprotein antigen coated to the wells.
- After a wash step, an anti-gp 51 monoclonal antibody (Mab) peroxidase conjugate is added. It fixes onto the free gp 51 sites and forms a complex: [Ag gp 51] - [Anti gp 51 Mab/peroxidase].
- Excess conjugate is then eliminated by a wash step. The enzyme coupled to the conjugate is revealed by addition of a substrate which is transformed into a coloured product. The corresponding optical densities are measured and interpreted as a function of threshold obtained from the controls.
 - In the absence of antibodies in the sample, an intense coloured reaction is seen due to the transformation of the enzyme conjugate bound to the free gp 51 sites fixed on the solid phase.
 - In the presence of anti-gp 51 antibodies in the sample, less enzyme conjugate is bound to the gp 51 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 Bovine Leukosis virus gp 51. | Use within 4 weeks after opening of the sachet which must be closed after use. |
| Conjugate (CJ) Mab anti-gp51 / peroxidase (concentrated 10X) | Dilute 10 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.

Reference : SBLV1.NA version n°12 – 24/01/07
Version n°11 → n°12: modification of part IV

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 decanted single sera or pooled sera (pools of up to 10 samples). 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 to be tested. 1:10 dilution can be performed either beforehand in hemolysis tubes, in blank microplates 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 previously diluted (ten-fold) serum samples per well. 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 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 200 µl of CJ diluted 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 either for 1 hour ± 5 min at + 5°C ± 3°C or for 30 min ± 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:

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 OD of the negative control (N) is > 0.500 , and
- the competition percentage of the positive control (P) is > 80 %.

This percentage can be calculated in the following way:

$$\% \text{ Sample} = \frac{\overline{\text{OD}} \text{ N} - \overline{\text{OD}} \text{ sample}}{\overline{\text{OD}} \text{ N}} \times 100$$

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Two methods for the calculation and interpretation are possible:

FIRST METHOD : CALCULATION OF THE COMPETITION PERCENTAGE (% Sample)

For each sample:

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

$\overline{\text{OD}}$: Mean optical density if the test is performed in duplicate.

Any serum sample presenting a competition percentage (% Sample) ≥ 50 % is considered as positive.

Any serum sample presenting a competition percentage (% Sample) < 30 % is considered as negative.

Any pool of sera presenting a competition percentage (% Sample) ≥ 30 % is considered as positive.

Any pool of sera presenting a competition percentage (% Sample) < 30 % is considered as negative.

Doubtful Zone: Any individual serum sample which presents a competition percentage situated in a zone from 30 to 50 % 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.

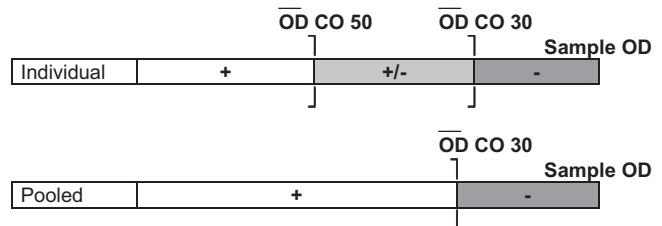
SECOND METHOD: ANALYSIS OF OPTICAL DENSITIES

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

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

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

Result interpretation:



Should you have any question, please contact us :
SYNBIOTICS EUROPE - 2 rue Alexander Fleming
69367 LYON Cedex 07 – France
Tel : +33 4.72.76.11.11 - Fax : +33 4.72.76.11.10
www.synbiotics.fr info@synbiotics.fr

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