

## LACTELISA<sup>®</sup> BLV Ab Mono Indirect

### KIT FOR THE DETECTION OF ANTI-gp 51 BOVINE LEUKOSIS VIRUS ANTIBODIES (BLV) IN TANK MILK

#### INDIRECT IMMUNOENZYMATIC TECHNIQUE

384 single well reactions

#### I. PRINCIPLE OF THE TEST

The LACTELISA<sup>®</sup> BLV Ab Mono Indirect kit uses an indirect immunoenzymatic technique allowing the detection of antibodies against enzootic bovine leukosis virus (BLV) envelope glycoprotein (gp51) in tank milk. Used as an initial diagnostic tool, this kit allows a screening of pooled milk samples. All positive results should be confirmed with a double well kit (LACTELISA<sup>®</sup> BLV Ab Bi Indirect from Synbiotics: ALBLV2). The reaction is composed of three steps:

1. Each milk sample is placed into a well strip sensitised with the BLV envelope glycoprotein, gp51. Antibodies (Ab) present in the sample fix to the viral antigen bound in the well.

2. After a wash step, an anti-bovine IgG monoclonal antibody (Mab) conjugate coupled to peroxidase is added. It fixes to the bovine immunoglobulins (antibodies) previously captured, forming a complex:  
(Ag gp51) - (Ab anti-gp51) - (Mab anti-bovine IgG/peroxidase)

3. Excess conjugate is eliminated by a 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 reaction, the optical densities are measured. The presence or absence of antibodies is determined by using a threshold value 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 the gp51 of the BLV.	Use within 4 weeks after opening of the sachet which must be closed after use.
Conjugate ( <b>CJ</b> ) (concentrated 20X) Mab anti-bovine IgG/peroxidase	Dilute 20 times in the conjugate diluent and use within 24 hrs after dilution.
Buffered peroxidase substrate ( <b>PS</b> )	Ready-to-use.
Negative control ( <b>N</b> )	Ready-to-use.
Positive control ( <b>P</b> )	Ready-to-use.
Wash solution ( <b>W</b> ) (10X concentrated)	Dilute 10 times in distilled or demineralised water. Use within 48 hrs after dilution.
Conjugate diluent ( <b>CD</b> )	Ready-to-use.
Stop solution ( <b>S</b> )	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:

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 reaction is performed on non-diluted tank milk (pools up to 50 milks), skimmed rather than unskimmed whole milk. Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Skimmed or unskimmed pooled milk	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 samples to be tested.  
The milk is tested without dilution. Nevertheless, it is recommended:
  - for skimmed milk samples, to homogenise well before pipetting.
  - for whole milk samples, to wait until decantation and pipette just underneath the lipidic layer.

## B. TEST PROCEDURE

### I - CONTROL AND SAMPLE DISTRIBUTION

#### 1. Control distribution:

Controls are ready-to-use.

After shaking the vials, dispense 200 µl of negative control (N) to wells A1 and A2, then dispense 200 µl of positive control (P) to wells B1 and B2.

#### 2. Sample distribution:

Dispense 200 µl per well of non diluted milk sample to be tested.

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 for 1 hour ± 5 min at +37°C ± 3°C or 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.

In the case of whole milks, it is recommended to use the diluted wash solution slightly warmed up to +37°C ± 3°C and 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 concentrate (CJ) 1:20 in the conjugate diluent (CD). (4 ml are needed for one strip, meaning 200 µl of CJ diluted in 3.8 ml of CD).

#### 2. Distribution of conjugate:

Add 200 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 +37°C ± 3°C or for 90 min. ± 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 200 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 optical density obtained with the positive control ( $\overline{OD} P$ ) is  $\geq 0.300$ , and
- the optical density obtained with the negative control ( $\overline{OD} N$ ) is  $< 0.75 \times OD P$ .

## VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

Two methods for calculating and interpreting the results are possible:

### First method : SAMPLE INDEX CALCULATION

Sample index =  $0.5 (\overline{OD} \text{ sample} - \overline{OD} P)$

$\overline{OD}$  = average of the ODs for the samples tested in duplicate.

Any tank milk presenting an index  $\geq 0$  is considered as positive.  
Any tank milk presenting an index  $< 0$  is considered as negative

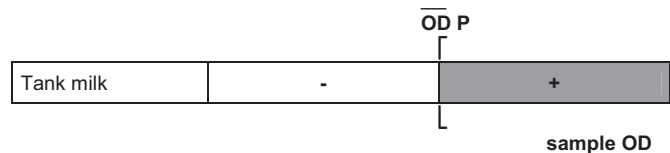
### Second method : ANALYSIS OF THE OPTICAL DENSITIES

$\overline{OD}$  threshold value:  $\overline{OD} P$

Compare each of the sample ODs to this threshold value

Note: Positive results should be confirmed with a double well ELISA kit (Synbiotics ref.: ALBLV2).

### Result interpretation :



Should you have any question, please contact us :  
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