

LACTELISA® BLV Ab Bi Indirect tank 250

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

INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 double well reactions

I. PRINCIPLE OF THE TEST

The LACTELISA® BLV Ab Bi Indirect tank 250 kit uses a double well indirect immunoenzymatic technique allowing the detection of anti-envelope glycoproteins (gp 51) antibodies in individual or pooled-milk samples up to 250.

The reaction is composed of three steps:

- Each milk sample to be tested is placed into two adjacent wells sensitised with a cellular antigen (odd columns) and a viral antigen (even column). Antibodies in the sample fix to the antigens coated in 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 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. 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 controls.

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) Mab anti-bovine IgG/peroxidase	Dilute in the conjugate diluent and use within 24 hrs after dilution.
Buffered peroxidase substrate (PS)	Ready-to-use.
Negative control (N)	To be diluted at 1:2 in the SD. Use within 24 hrs after dilution.
Positive control (P)	To be diluted at 1:2 in the SD. Use within 24 hrs after dilution.
Milk 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 reaction is performed on either individual milks or pooled (tank) milks (up to 250).

Milk samples are tested at a 1:2 dilution in the milk sample diluent. 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 (20°C)
Individual or pooled (tank) 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, each plate.

A. PRELIMINARY STEPS

- Carefully set up the distribution and identification of controls and samples.
- Prepare the samples to be tested.
1:2 dilutions should 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:

The controls are not ready-to-use and must be diluted as samples to be tested. After shaking the vials, distribute 100 µl of milk sample diluent (SD) and 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 200 µl of the two-fold diluted milk sample in two adjacent wells (cellular and viral antigens).

- Dilute directly in the wells: add 100 µl of milk sample diluent plus 100 µl of milk sample in two adjacent wells.
- 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

I. Only pooled samples up to 100 milks

Short incubation protocol: 1 hour ± 5 min at +37°C ± 3°C (1:20 conjugate dilution) or

Long incubation protocol: overnight (14-18 hours) at +5°C ± 3°C (1:30 conjugate dilution).

II. Individual samples and pooled samples whatever the pool size (up to 250 milks)

Short incubation protocol: 1 hour ± 5 min at +37°C ± 3°C (1:20 conjugate dilution)

WASHING:

Wash buffer: dilute 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 use the diluted wash solution 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:

The conjugate must be diluted according to the protocol used. Prepare the conjugate solution by diluting the concentrate (CJ) either 1:20 (short incubation protocol) or 1:30 (long incubation protocol) in the conjugate diluent (CD). 4 ml are needed for one strip, meaning either 200 µl of CJ in 3.8 ml of CD or 133 µl of CJ in 3.87 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:

I. Only pooled samples up to 100 milks

Short incubation protocol: 1 hour ± 5 min at +37°C ± 3°C (1:20 conjugate dilution) or

Long incubation protocol: 90 min ± 5 min at +20°C ± 5°C (1:30 conjugate dilution).

II. Individual samples and pooled samples whatever the pool size (up to 250 milks)

Short incubation protocol: 1 hour ± 5 min at +37°C ± 3°C (1:20 conjugate dilution).

WASHING:

Carefully remove the adhesive film and wash 6 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 differences in the optical densities obtained with the positive control are ≥ 0.300 , and
- the differences in optical densities obtained with the negative control are $< 0.20 \times \Delta OD P$

VIII. EXPRESSION AND INTERPRETATION OF THE RESULTS

The presence of anti-bovine leukosis virus gp 51 antibodies is determined by the difference in the optical densities (ΔOD) 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 index for each sample tested as follows:

Sample Index = $0.25 \times (\Delta OD \text{ Sample} - \Delta OD P)$

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

Any individual milk sample showing an index $\geq -0.125 \times (\Delta OD P)$ is considered as positive.

Any individual milk sample showing an index $< -0.20 \times (\Delta OD P)$ is considered as negative

Any individual milk sample showing an index between $-0.125 \times (\Delta OD P)$ and $-0.20 \times (\Delta OD P)$ is considered as doubtful

Any pool of milk sample showing an index $\geq -0.20 \times (\Delta OD P)$ is considered as positive.

Any pool of milk sample showing an index $< -0.20 \times (\Delta OD P)$ is considered as negative.

Any individual milk sample presenting a result situated in a DOUBTFUL ZONE between $-0.20 \times (\Delta OD P)$ and $-0.125 \times \Delta OD P$ should be re-tested.

If the doubtful result persists, a new control should be performed on a different sample from the same source.

■ Any individual or pool of milk presenting an OD value on the cellular Ag well higher than $1.5 \times \Delta OD P$ should not be submitted to interpretation. Such samples should be retested using the final 1:4 dilution.

■ When using pooled milks including between 2 and 50 milks, it is advised to use as cut off value the index $-0.125 \times \Delta OD P$.

Method 2 : ANALYSIS OF THE OPTICAL DENSITIES

Calculate the ΔOD thresholds corresponding to the $(0.50 \times \Delta OD P)$ and the $(0.20 \times \Delta OD P)$

Compare each of the sample ΔOD s to these threshold values.

Result interpretation :

	$0.20 \times (\Delta OD P)$	$0.50 \times (\Delta OD P)$	
Individual milk	-	+/-	+
			sample OD
Pool of milks *	-	$0.50 \times (\Delta OD P)$	+
*(up to 50 samples)			sample OD
Pool of milks**	-	$0.20 \times (\Delta OD P)$	+
** (pool size > 50 samples)			sample OD

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
 FOR VETERINARY USE ONLY /
 FOR IN VITRO USE ONLY