SYNBIOTICS CORPORATION

SYNBIOTICS EUROPE SAS
2, rue Alexander Fleming
F- 69367 Lyon, Cedex 07

LACTELISA® BLV Ab Bi Indirect

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

INDIRECT IMMUNOENZYMATIC TECHNIQUE

192 double well reactions

I. PRINCIPLE OF THE TEST

The LACTELISA® BLV Ab Bi Indirect kit uses a double well indirect immunoenzymatic technique allowing the detection of anti-envelope glycoproteins (gp 51) antibodies in milk. The reaction includes three steps:

1. Each milk sample to be tested is placed into two adjacent wells sensitised with a cellular antigen (odd columns) and a viral antigen: gp51 (even column). Antibodies in the sample fix to the antigens coated in the viral antigen well.

2. Following a wash step, an anti-bovine IgG monoclonal antibody (Mab) peroxidase conjugate is added. It fixes onto the antibodies present, forming a complex:
(gp 51 Ag) - (anti-gp 51 Ab) - (anti-bovine IgG Mab - peroxidase).

3. 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 20X) anti-bovine IgG Mab/peroxidase	Dilute 20 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.
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 mentionned 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 individual or pooled (up to 50 milks), skimmed rather than unskimmed milks. Samples should be stored as follows:

Samples	Cold (+ 5°C)	Freeze (- 20°C)	Lab Temperature (20°C)
Individual or 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, add 200 µl of negative control (N) to wells A1 through A4 (A1 & A3: cellular antigen; A2 & A4: viral antigen).
Add 200 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 non diluted milk sample in two adjacent wells (cellular and viral antigens). The 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℃ ± 3℃
or overnight (14-18 hours) at +5℃ ± 3℃.

WASHING:
Wash buffer: dilute the concentrated washing solution (W) 1:10 in distilled or demineralised water.
In the case of whole milk, it is recommended to use the diluted wash solution slightly warmed up to +37℃ ±3℃ and incre ase, 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℃ ± 3℃
or for 90 min. ± 5 min at +20℃ ± 5℃.

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℃ ± 5℃), 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 ≥ 0.200, and
- the differences in optical densities obtained with the negative control are < 0.50 x ΔOD 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 (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 x (ΔOD Sample - ΔOD P)
(ΔOD P : the average of the differences of the optical densities observed for the positive control tested in duplicate).

Any individual milk sample showing an index ≥ 0 is considered as positive.

Any individual milk sample showing an index < -($\Delta\overline{OD}$ P) /16 is considered as negative.

Any individual milk sample showing an index between 0 and -($\Delta\overline{OD}$ P) /16 is considered as doubtful.

Any pool of milks sample showing an index ≥ -($\Delta\overline{OD}$ P) /16 is considered as positive.

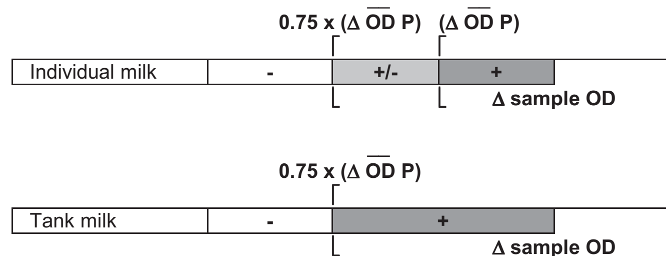
Any pool of milks sample showing an index < -($\Delta\overline{OD}$ P) /16 is considered as negative.

Doubtful zone:
Any individual milk sample presenting a result situated in a DOUBTFUL ZONE between 0 and -(ΔOD P) / 16 should be retested.
If the doubtful result persists, a new control should be performed on a different sample from the same source.

Method 2 : ANALYSIS OF THE OPTICAL DENSITIES

Calculate the $\Delta\overline{OD}$ thresholds corresponding to the ($\Delta\overline{OD}$ P) and the [0.75 x (ΔOD P)].
Compare each of the sample $\Delta\overline{OD}$s to the thresholds ($\Delta\overline{OD}$ P) and [0.75 x (ΔOD 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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