LT Biotech

Operating instruction

«LTELISA Brucella» indirect ELISA kit for detection antibodies to Brucella in animal milk

REF **LT-E-Milk-BRU-01s**
Version: 001-2017-04

General Description:

This Test kit is designed for detection of specific antibodies to *Brucella* in individual and bulk (pool) milk samples. Wells of polystyrene microwell strips are coated with *Brucella* LPS and any specific antibodies from individual and bulk milk samples are bound to immobilized antigen. Such antigen-antibody complexes are further detected with horseradish peroxidase (HRP)-labeled recombinant protein G, which binds to all IgG subclasses from animal milk. Addition of enzyme substrate (chromogen TMB) results in development of blue colored product in the case HRP-labeled conjugate is present in the well. Strong color development indicates the presence of IgG to *Brucella* LPS in the milk sample. Very weak or no color development indicates the absence of IgG to *Brucella* LPS in the milk sample.

This kit allows assaying of 920 individual or pool of ≤250 milk samples, without control samples. In Member States of the European Union, ELISA tests done on milk must be conducted on bulk (pool) milk samples as required in Annex C of EU Decision 2008/984/EC.

This kit allows assaying of 184 samples, without control samples.

Animal species: Wild and domestic mammals.

Kit Contents:

Component	Quantity
Antigen-coated plate	10 plates
Conjugate (x100)	1 vial, 1.5 ml
Wash solution concentrate(x30)	2 vials, 75.0 ml
Stop reagent	1 vial, 60.0 ml
Substrate Solution (TMB)	5 vials, 21.0 ml
Positive control (C+)	1 vial, 1.5 ml
Negative control (C–)	1 vial, 1.5 ml
Sticky film	10 piece
Operating instruction	1 piece

Preparation **of reagents (for 1 plate)**:

1. Warm up reagents at room temperature (18–22) ºC during 30 minutes.
2. Prepare (1X) Wash solution. Intensively shake Wash solution concentrate (15ml) and dilute with **435.0 ml** of distilled or deionized water. If Wash solution concentrate contains sediment dissolve crystals by heating at (35–37) ºC. Keep (1X) Wash solution at temperature (2–8) ºC no longer than 5 days in clean vial tightly closed.
3. Prepare conjugate solution. Conjugate solution (1X) has to be prepared before use. Dilute one part of Conjugate (x100) with 99 parts of Wash solution (1X) in a clean vial. Mix well avoiding foaming.
   For example:
   For one strip add 1.0 ml of Wash solution (1X) to clean bottle, and then add 0.01 ml of Conjugate (x100). Mix well by pipetting.
   For plate add 10.0 ml of Wash solution (1X) to clean bottle, and then add 0.1 ml of Conjugate (x100). Mix well by pipetting.
4. Prepare milk specimens. It is possible to test skimmed or whole milks. Skim milk: Centrifuge (15 min at 2000g) each whole milk sample, or let samples sit, so that the cream separates from the lactoserum (cream on the top, lactoserum on the bottom). The milk samples are transported as soon as possible to the lab, preferably within 24 hours. If longer transportation is needed it is highly recommended to use preservatives such as Bronopol. Preserved samples may be kept for several days at 4ºC.

Freeze at -20ºC for long term storage (for three months).

Do not freeze and thaw milk specimens twice. Milk samples with sodium azide, or bacterial contamination are not suitable for the analysis!

Test Procedure:

1. Remove protective foil from antigen-coated plate and add **0.05 ml** of 1X Wash solution into wells of the plate **except wells A1, B1, C1, D1. Into wells A1, B1, C1, D1 add 0.095 ml of** 1X Wash solution.
2. Add **0.05 ml** of individual or bulk (pool) milk samples into wells of the plate leaving free of the first 4 wells (A1, B1, C1, D1) for Positive and Negative controls.
3. Add **0.005 ml** of the Positive control (C+) into wells A1, B1, and **0.005 ml** of the Negative control (C–) into wells C1, D1. Carefully repipette the mixture in wells. Take care not to spill samples from well to well. Stick the plate by enclosed film and incubate at temperature **37.0ºC for one hour**.
4. Rinse the plates 4 times with **0.35 ml** of a 1X Wash solution using microplate washing machine: aspirate the well contents completely, fill up the wells at each rinse and aspirate completely. Make sure that no fluid remains on the strips and stripholder after the last aspiration. If necessary, dry the plate by tapping on absorbent paper.
5. Add **0.1 ml** of 1X Conjugate solution (see above) into each well. Stick a plate by enclosed film and incubate at temperature **37.0ºC for 30 minutes**.
6. Rinse the plates 6 times with **0.35 ml** of 1X Wash solution following washing procedure described in point #4.
7. Add **0.1 ml** of Substrate Solution (TMB) into each well. Incubate plate at **20–25^{0}C for 30 minutes**. Avoid leaving the plate in direct sunlight.
8. Stop colour reaction by adding 0,05 ml of Stop Solution to each well.
9. Measure the optical density (OD) of the controls and samples at 450/620 nm using a microplate reader. To prevent the fluctuation in OD values measure OD within 3 minutes after reaction was stopped.

Result interpretation:

1. Calculate the mean OD-values for each control (mean OD Negative Control and mean OD Positive Control).
2. Calculate the percent positivity (PP) values for negative control and samples, using the formula:

$$PP = \frac{Mean\ OD\ sample\ or\ Negative\ control}{Mean\ OD\ Positive\ control} \times 100$$

For example:

$$PP = \frac{0{,}299}{1{,}690} = 17.7\%$$

3. Analysis is considered correct, if mean value of OD Positive Control is greater than 1.0, mean value of PP Negative Control is less than 8.
4. Results of the analysis for investigated samples are considered **Positive** if PP of the samples are equal or greater than 10%**.** Results of the analysis for investigated samples are considered **Negative if PP of the samples are less than 10%.**
5. **10%** - the constant value, which is determined by the manufacturer and may be changed in different LOT numbers.

Storage and Stability:

Store all reagents at 2-8^{0}C. **Do not freeze**. Reagent will remain stable until the expiration date when stored as recommended. Do not use test kit beyond the expiration date printed on the label.