Operating instruction

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

REF **LT-E-BRU-02**
Version: 002-2017-05

General Description:

This Test kit is designed for detection of G isotype antibodies (IgG) to *Brucella* in samples of animal serum.

Wells of polystyrene microwell strips are coated with *Brucella* LPS and any specific antibodies from serum 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 serum. 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 serum sample. Very weak or no color development indicates the absence of IgG to *Brucella* LPS in the serum sample.
This kit allows assaying of 184 samples, without control samples.
Animal species: Wild and domestic mammals.

Kit Contents:

Component	Quantity
Antigen-coated plate	2 plates
Conjugate immunoenzyme (x100)	1 vial, 0.3 ml
Wash solution concentrate(x30)	2 vials, 15.0 ml
Stop reagent	1 vial, 12.0 ml
Serum dilution buffer	2 vials, 12.0 ml
Substrate Solution (TMB)	1 vial, 21.0 ml
Positive control (C+)	1 vial, 0.1 ml
Negative control (C–)	1 vial, 0.1 ml
Sticky film	2 pieces
Operating instruction	1 piece

Preparation of reagents:

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 serum specimens. Samples containing aggregates and/or precipitate should be clarified by centrifugation for 10 minutes at 2500-3000 RPM. Serum samples may be stored at (2-6) °C no longer than three days. It is allowed to store the serum samples at - 20°C (or lower) for three months and at -70 °C for two years. Do not freeze and thaw sera specimens twice. Sera with sodium azide, hemolysis, hyperlipidemia or bacterial contamination are not suitable for the analysis!

LT BIOTECH LTD
Lithuania, Vilnius
Mokslininku 6A, LT-08412
Tel/fax +370 5 216 02 27
info@ltbiotech.lt
www.ltbiotech.lt

Test Procedure:

1. Remove protective foil from antigen-coated plate and add **0.095 ml** of Serum dilution buffer into all wells of the plate.
2. Add **0.005 ml** of serum 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. During the pipetting solution in wells may be changed in color. 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:

$$PP = \frac{0{,}299}{2{,}690} \times 100 = 11.1\%$$

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.

LT BIOTECH LTD
Lithuania, Vilnius
Mokslininku 6A, LT-08412
Tel/fax +370 5 216 02 27
info@ltbiotech.lt
www.ltbiotech.lt