

Brucella qPCR kit

Cat: LTQPCR-BRU

Quantity 50 tests

1. Purpose

Brucellosis is a bacterial disease caused by various *Brucella* species, which mainly infect cattle, swine, goats, sheep and dogs. Humans generally acquire the disease through direct contact with infected animals, by eating or drinking contaminated animal products or by inhaling airborne agents. As a consequence, brucellosis is classified as the most important zoonotic disease by WHO.

The «Brucella qPCR kit» is intended to detect brucella DNA in serum / plasma / blood / milk, animal tissue, feed, food and environmental samples, which is rapid, accurate and easy-to-operate.

The test kit «Brucella qPCR kit» is designed for multiplexed detection of the specific to pathogenic Brucella DNA (bcsp 31 gene), according FDA validated protocol, and Internal Control (Ruminant gene) as sample processing control by real time polymerase chain reaction (PCR) with fluorescent detection of the analysis results.

2. Clinical significance

Brucella are small, Gram-negative coccobacilli that act as facultative intracellular pathogens, producing chronic infections that often persist for life. Four species are known to infect humans: *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis*. Human brucellosis is most commonly associated with consumption of unpasteurized milk and soft cheeses prepared from the milk of infected animals, particularly goats carrying *B. melitensis*, as well as occupational exposure among veterinarians, farmers, and slaughterhouse workers.

3. Principle of analysis

The method is based on the isolation of total DNA from samples, and further amplification of specific regions of Brucella DNA (bcsp 31 gene) using specific oligonucleotide primers and the synthesis of complementary DNA chains using the enzyme Hot start Taq-DNA polymerase. Fluorescent detection of the analysis results is performed both in real-time (Real-time mode) or end point assay using two fluorescence detection channels (FAM525 nm, ROX580 nm), table 1.

Table 1. Target specificity

Marker	Detection channel
Brucella, bcsp 31 gene	FAM
Internal control, ruminant gene	ROX

4. Kit contents:

Table 2. PCR reagents:

Component name	Quantity	Volume
Brucella Master Mix (Lyophilized)	1 vial	
Brucella Positive Control (Lyophilized)	1 tube	

5. Storage and stability

The test kit reagents are stable for the shelf life indicated on the label (at least 12 months from manufacture date) if stored at room temperature.

6. Sample preparation

Serum / plasma / blood / milk samples can be used directly for Nucleic Acids extraction. Food, feed animal tissues can be processed according commercially available DNA extraction kit recommendations. We recommend using our Nucleic acids extraction kit compatible with automatic Nucleic extraction device LTEXT1.

7. Reagent preparation

Master Mix preparation: Add distilled water 0,5 mL directly to Lyophilized Master Mix, wait 5 min, mixing well and dispense 10 µL into PCR testing tubes.

Positive control preparation: Add distilled water 0,5 mL directly to Lyophilized Positive Control, wait 5 min, mixing well and use directly 10 µL for PCR or use 100 µL for Nucleic acid extraction (to control Nucleic acid extraction efficiency). Reagents and controls must be kept in a chilled stand. After use reagents and controls can be freeze at minus 20°C for storage. Several freezing-thawing cycles are allowed.

8. Assay procedure

8.1. Select the required amount of the PCR tubes or wells in the PCR plate.

8.2. Using pipette tips with an aerosol filter dispense Master mix 10µL add 10 µL of DNA samples (Positive and Negative control samples).

8.3. Place testing tubes in the fluorescence PCR instrument, and set the negative/positive control and unknown samples parameters for PCR reaction according to the instrument operating instructions. Fluorescence is measured at 60°C on the FAM, and ROX channels. The PCR parameters are shown in the table 3:

Table 3. PCR run method parameters

Step	Temperature	Time	Repeats	Data collections
1	95 C	5 min	1	No
2	95 C	10 sec	45	No
	60 C	30 sec		Yes

8.6. Start run.

9. Result records and their interpretation is shown in the table 4:

Table 4. PCR results interpretation

Marker	Fluorescent channels	Brucella Positive	Brucella Negative
Brucella bcsp 31 gene	FAM	Ct≤ 45	Undetectable
Internal control Ruminant gene	ROX	Ct≤ 35	Ct≤ 35