PCR KIT FOR DETECTION OF Brucella spp. DNA

(YzzyTest Brucella spp.)

APPLICATION MANUAL

1. Kit Content

Cat no	Content
Brucella spp. E-55	**1. PCR-reagent** 1 tube with black cap (includes enzyme in buffer) – 0.55 ml
	2. Brucella Primers 1 tube with yellow cap (includes primers and probe) – 0.55 ml
	3. Reagent "DNA-express" 1 tube with violet cap (includes special sorbent in buffer) – 1.37 ml
	4. External control (EC) 1 tube with green cap – 0.55 ml
	5. Positive control (PC) 1 tube with red cap – 0.5 ml
	6. Negative control (NC) 2 tubes with white caps – 1 ml

2. Intended Use

Brucella spp. Kit (E-55 format) is used for the detection of Brucella spp. DNA in serum, plasma, vaginal swab, tissue samples from animals using real-time PCR instruments.

3. Storage

The components of the Brucella spp. PCR Kit could be stored at +2 - +4°C for up to 12 months from the date of manufacturing without reduction in sensitivity.

4. Introduction

Brucellosis - also called Bang's disease, Crimean fever, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, rock fever, or undulant fever - is a zoonotic infection of domesticated and wild animals, caused by bacteria of the genus Brucella.

Brucella are gram-negative, intracellular bacteria that can infect many species of animals including wildlife species and marine mammals, as well as humans. Species infecting domestic livestock are B. melitensis (goats and sheep), B. suis (pigs), B. abortus (cattle and bison), B. ovis (sheep), and B. canis (dogs). Humans become infected by ingestion of food products of animal origin (such as undercooked meat or unpasteurized milk or dairy products), direct contact with infected animals, or inhalation of infectious aerosols. Transmission from human to human is rare but possible.

The Brucella spp. PCR Kit is a highly sensitive and specific solution for the detection of Brucella spp. DNA in samples from infected animals. The express method of DNA purification from the wide range of samples allows detecting the pathogen early and easily.

5. Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected using fluorescent dyes. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward. The Brucella spp. PCR Kit contains all of the necessary reagents for the detection of Brucella DNA, including reagents for Express DNA purification, external, positive and negative controls. An external control excludes the possibility of false-negative results.

The kit uses two specific primer/probe combinations: First - for Brucella DNA yielding HEX™ fluorescence and second - for an external control yielding FAM™ fluorescence. A PC serves to verify the functionality of the reaction mixture for the amplification of the Brucella DNA target.

6. Equipment and reagents to be supplied by user

-Pipets (0.5 µl – 1000 µl);
-Nuclease-free aerosol-resistant pipet tips with filters;
-Sterile 1.5 ml tubes;
-Benchtop centrifuge with rotor for 1.5 ml tubes (RCF ≥ 10,000 × g);
-Vortex mixer;
-Disposable gloves, powderless;
-Biohazard waste container;
-Refrigerator and freezer;
-Tube racks;
-Real-time PCR machine (e.g. Rotor-Gene 3000, 6000, Q; Biorad CFX 96; Agilent MX 3005P and etc.);
-PCR tubes, suitable for used PCR machine.

While working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

7. Sample preparation

-Plasma and serum samples are used without special preparation with "DNA Express reagent"

- The swabs are suspended in sterile PBS and purified with "DNA Express reagent".

-Tissue and organ samples should be prepared as 10% suspension (5-20% range is allowed) in phosphate buffer saline (PBS) and then homogenized using porcelain mortars and pestles or automatic homogenizers (recommended). Samples after homogenization are left in a rack for 5 minutes to sediment large particles. DNA is isolated from the supernatant with "DNA Express reagent".

-To isolate DNA from semen, environmental samples, diary products and enrichment cultures we recommend to use "YzzyRNA extract" kit.

8. Protocol for DNA purification using the reagent "DNA-express"

1. Prepare 1.5 ml or 2 ml microcentrifuge tubes in count of tested samples but adding one extra tubes for NC extraction. Mark the tubes carefully.

2. Thoroughly mix the reagent "DNA-express" by vortexing before use.

3. Pipet 25 µl of the reagent "DNA-express" into each tube. Then add 5 µl of EC into each one.

3. Add 25 µl of the test material (plasma, serum, tissue extract, swab) into the corresponding tube.

4. Add 25 µl of NC into a separate tube and mark it as Negative Control of Extraction (NCE).

5. Close the reaction tubes with the corresponding caps. Pulse-vortex tubes for approximately 5 seconds.

6. Incubate at 95°C for 10 min with constant mixing (more than 1000 rpm recommended).

7. Centrifuge for 5 min at minimum 10,000 × g.

8. Carefully transfer the supernatant (about 30 µl) into the marked 1.5 ml microcentrifuge tubes. Store the extracted DNA at –20°C until required for PCR amplification procedure.

9. Protocol for real-time PCR detection of Brusella spp.

1. Before use mix reagents by inverting tubes several times and spin the reagents briefly.

2. In a 1.5 ml microcentrifuge tube prepare the PCR reaction "Master Mix" in bulk for the number of samples to be assayed but allowing for one extra sample:

(10 µl PCR-reagent + 10 µl Brucella Primers)*(N+1),

where N is number of samples.

3. Pipet 20 µl of the "Master Mix" into each reaction tube. Then add 10 µl of the sample DNA (Table 1). Include positive and negative control reactions.

Positive Control: Use 10 µl of the PC instead of sample DNA.

Negative Control: Use 10 µl of the NC instead of sample DNA.

Table 1. Preparation of reaction mix

Component	Volume
Master Mix	**20**
Sample	**10**
Total volume	**30**

4. Close the reaction tubes with the corresponding caps.

5. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Brucella/External control	Reporter
Brucella spp.	HEX/Yellow
External Control	FAM/Green

6. Run the real-time PCR protocol according to Table 3.

Table 3. PCR protocol

Temperature	Time	Number of cycles
95°C	15 min	1
95°C	5 s	10
60°C	10 s	
67	10 s	
95°C	5 s	35
60°C	10 s, Fluorescence data collection (FAM/GREEN; HEX/YELLOW)	
67	10 s	

10. Interpretation of Results

For the assay to be valid, the PC must give a signal in both the FAM and HEX channels with a Ct ≤27. The NC and NCE must give no signal in HEX channel.

The possible results of control samples are also summarized in Table 4.

Table 4. Evaluation of control samples

Controls	FAM	HEX
NCE	Ct≤27	No Signal
NC	No Signal	No Signal
PC	Ct≤27	Ct≤27

The following results are possible if working with unknown samples (Table 5).

The sample is positive for Brucella spp., and the assay is valid, if the following criteria are met:

–The sample yield a signal in HEX channel;

–The NC and NCE does not yield a signal in the HEX channel.

The sample is negative for Brucella spp., and the assay is valid, if the following criteria are met:

– The sample yields a signal in the FAM channel but not in the HEX channel;

–The NC and NCE does not yield a signal in the HEX channel.

The sample results are inconclusive, and the assay is invalid, if the following occurs:

– The sample yields no signal in the FAM and HEX channels.

Table 5. Results interpretation table.

Sample result	FAM (EC)	HEX (pathogen)
Brucella spp. positive	+/-	+
Brucella spp. negative	+	–
Inconclusive	–	–

11. Troubleshooting

Trouble	Possible causes	Solution
No signal for the EC	PCR inhibition	-dilute the DNA 1:5 in nuclease free water; -repeat the whole test procedure starting with new sample material; -use another DNA extraction method
No signal for the PC	incorrect preparation of the reaction mixture	carefully prepare new reaction mixture
	incorrect cycling conditions	carefully set cycling conditions
	improper storage	use properly stored kit
NC signal presence	contamination	-perform decontamination procedures; - use new kit

AUTHORIZED BY
YZZY Biotech
R&D laboratory