AMD Ltd Zena Max Brucella spp. qPCR Detection Kit

CE IVD

REF **KD485648-100**

Advanced Molecular Diagnostics Ltd is a diagnostics company specialising in the manufacture and supply of molecular biology instruments, reagents and consumables.

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Intended Use

This assay is an in vitro PCR test for the qualitative identification of *Brucella* spp. DNA in human clinical samples, such as whole blood, serum, or other validated specimen types, using a hydrolysis-probe detection method in a highly sensitive qPCR kit.

This product is for research use only.

Overview

Brucella spp. are small, Gram-negative, facultative intracellular bacteria responsible for brucellosis, a zoonotic infection transmitted primarily through consumption of unpasteurised dairy products or direct contact with infected animals or their secretions [1,2]. Human disease typically presents with non-specific symptoms such as fever, sweats, malaise, arthralgia, and hepatosplenomegaly, while chronic or relapsing forms may involve osteoarticular, neurological, or cardiovascular complications [1,3]. Because Brucella can persist within macrophages and cause prolonged or insidious illness, early and accurate laboratory diagnosis is essential for effective treatment and public-health control [2]. Molecular detection by PCR provides a rapid and highly specific method for identifying Brucella DNA, complementing serological and culture-based testing, the latter of which may require extended incubation and carries laboratory biosafety risks [3,4].

Principles of the Test

The qPCR kit is designed for the detection of *Brucella spp.* by the real-time Polymerase Chain Reaction (PCR) method. The *Brucella spp.* detection is based on the amplification of a specific conservative DNA sequence and measuring the amplification product concentration using PCR process and fluorophore labelled probes. *Brucella spp.* presence is indicated by the FAM fluorophore fluorescence growth. For the DNA isolation quality control and possible PCR inhibition control there are primers and probe for internal control gene amplification present in the reaction mix. Amplification of internal control gene is indicated in the HEX fluorophore fluorescence channel. The detection kit utilizes the “hot start” technology, minimizing non-specific reactions and assuring maximum sensitivity. The ready-to-use Master Mix contains uracil-DNA-glycosylase (UDG), eliminating possible contamination of the PCR reaction by amplification products. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

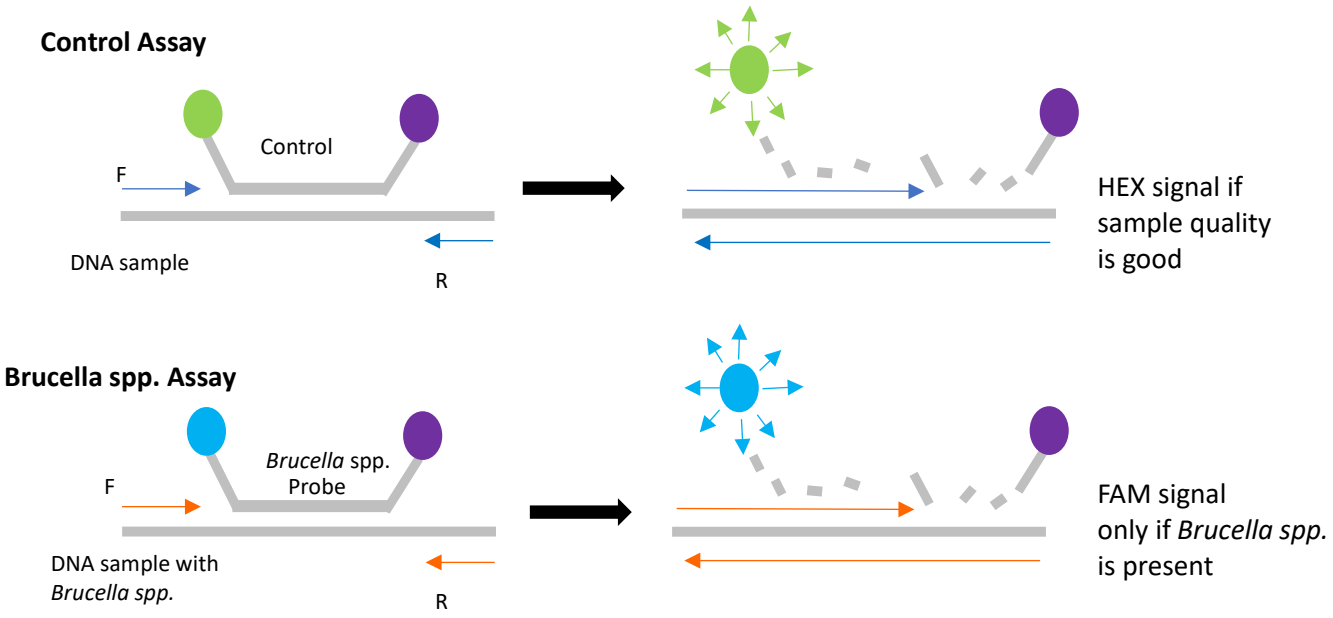

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of *Brucella spp.* The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The *Brucella spp.* assay will produce a FAM signal, if *Brucella spp.* is present, however if it is not present, no FAM signal will be detected. Due to assay competition, the HEX signal may be reduced or absent when the FAM signal is strong.

Materials Provided

Kit content

Item	
Brucella spp. qPCR Master Mix	2 x 1 ml
Brucella spp. Positive Control	1 x 0.05 ml
Nuclease-free Water	1 x 1 ml

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -15 °C and -25 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept. Repeated freezing and thawing of the kit components may result in lower detection quality. It is recommended that the master mix is aliquoted to avoid this. Avoid exposure to light. Ensure that all reagents are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (but not provided)

DNA Extraction: AMD manufactures the LUCO AMD NA Extraction Kit which can be used for isolating DNA from the samples. Other leading brands of IVD DNA extraction kit are acceptable for use with this diagnostic kit. If using any other kit, please validate for use with this assay before proceeding with sample testing.

PCR Instrument: AMD manufactures a high standard real-time qPCR system which is recommended for use with this kit. This kit can also be used with all other qPCR systems.

Consumables: AMD manufactures high quality nuclease and pyrogen free PCR plastic ware suitable for use with this kit. Use of other manufacturers' consumables is also acceptable.

Other Laboratory Equipment: Vortex, micro centrifuge, micro pipettes and tips, microfuge tube rack, PCR tube/plate rack, spectrophotometer.

Warnings and Precautions

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). Discard sample and assay waste according to your local safety regulations. It is essential to follow the instructions in this manual precisely, to ensure the best product performance.

Sample Collection, Storage and Transport

We advise using blood, serum and other validated samples. Samples are stored for a maximum of 6 hours at 2-8^{0}C before sample preparation. Please ensure that the sample is stored correctly and kept away from any contamination. Aliquot and store the samples at -20^{0}C or -80^{0}C immediately if they are not to be used within this time-period. Freeze thawing may compromise the test results. Ensure that samples are stored correctly and kept away from any contamination. For transportation, the samples should be placed in a shatterproof transport container to avoid the potential danger of infection due to sample leakage. Transport samples following the local and national instructions for the transport of pathogenic material, by courier, if possible, at a temperature of 2-8^{0}C and no longer than 6 hours following collection.

Assay Procedure

Sample Preparation

For optimal results use the LUCO AMD Nucleic Acid Extraction Kit to extract DNA from the sample. It is important to ensure that all samples are kept free from any contamination and correct storage procedures are followed to ensure there is no damage to the DNA. Store the DNA at 2-8°C for up to 24 hours, then at -20°C for longer term storage to ensure there is no damage to the DNA.

PCR Set up

1. Ensure that all reagents and samples are thawed completely, mixed and briefly centrifuged. Keep all reagents and samples on ice during this procedure.
2. Set up the reactions using the table below.

Product	Volume
AMD Brucella spp. PCR M. Mix	20µl
DNA Sample	5µl

3. Add the DNA samples and control to the PCR tubes/plate. Also add 5µl nuclease free water in place of the DNA as a No Template Control (NTC).
4. Seal the PCR tubes or plate and briefly spin to ensure that the reagents are at the bottom and no air bubbles are present.
5. Place the plate/tubes in the qPCR thermal cycler and use the following thermal profile:

Thermal Profile

Stage/Step	Temperature	Time
Stage 1: Step 1	30°C	2 mins
Stage 1: Step 2	95°C	2 mins
	40 Cycles	
Stage 2: Step 1	95°C	10 secs
Stage 2: Step 2*	58°C	30 secs

*Data collection step in FAM (diagnostic assay) and HEX (internal control assay) channels.

6. When the run has finished, dispose of the PCR reaction tubes/plate in an appropriate manner in accordance with local and national regulations.

Data Analysis

Analyse the data if the software does not do this automatically at the end of the run. Export the data to Excel or a PDF report, depending on the qPCR instrument used, and view the results.

Interpretation of Results

This is a qualitative assay and the detection of *Brucella spp.* in a sample is calculated by reference to the standard curve. The results should be interpreted as follows, using Table one as a quick reference guide:

- The internal control assay signal in the HEX channel should be present but may be absent or have a high Cq value (low signal) when the diagnostic assay signal is strong.
- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for **for *Brucella spp.***
- If there is a HEX signal but no FAM signal, the sample is **negative** for ***Brucella spp.***
- If there is no signal in either channel, or only within the FAM channel, the result is **inconclusive**.

Result		**Interpretation**
HEX (IC)	**FAM (Brucella spp.)**	
Positive (Ct<34)	Positive (Ct<38)	Positive for Brucella spp.
Positive (Ct<34)	No Cq	Negative for Brucella spp.
No Cq	Positive (Ct<38)	Positive for Brucella spp.
No Cq	No Cq	Inconclusive

Table 1. Interpretation of the results obtained from the Pheno Brucella spp. qPCR Kit.

Product Limitations

This kit is for in vitro diagnostic procedures and should only be used by specifically trained laboratory personnel. The expiry date of all components must be checked before use and disposed of if expired. Occasionally mutations may arise in the genomic region targeted by the primers and probes of this

this assay, leading to reduction in performance or failure of the assay. The assay design and efficacy are reviewed periodically.

Additional Information

AMD produces real-time PCR kits with a wide range of applications for researchers from gene expression analysis, cDNA, and population genotyping studies, to the multiplex detection of several disease targets real-time PCR with excellent sensitivity and specificity. Please familiarise yourself with the qPCR instrument before using the AMD Brucella spp. PCR Kit.

AMD PCR software offers several analysis modules, including quantification, melt curve, gene expression, allelic discrimination, and end-point analyses. A data file is automatically generated after a run with the gene expression module which allows you to easily view up to six different charts or tables, such as the amplification plot, standard curve, gene expression chart, plate layout, or melt peak with the Custom Data View tab. The software used will show screens including a Cq reading and the linearity of the samples used.

References

1. World Health Organization. Brucellosis in humans and animals. WHO Guidance. 2006.
2. Centers for Disease Control and Prevention (CDC). Brucellosis: General Information. Updated 21 February 2024.
3. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *N Engl J Med.* 2005;352:2325–2336.
4. Navarro E, Segura JC, Castaño MJ, Solera J. Use of PCR in the diagnosis of human brucellosis: a systematic review. *Clin Infect Dis.* 2006;42(9):1266–1273.

Contact

If you have any queries, comments or complaints please refer to our website at:
www.am-diagnostics.co.uk info@am-diagnostics.co.uk

Harmonised Symbols

The following is a key of all harmonised symbols used by AMD Ltd (Advanced Molecular Diagnostics) in Instructions for Use (IFUs) and product labelling.

Symbol	Definition	Details
	Manufacturer name and address	AMD Ltd BioCity Nottingham, Pennyfoot Street, Nottingham NG1 1GF United Kingdom
	UDI-DI number for the product given	Basic: 506105998BRUCBR UDI-DI: (01)05061059980687 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD485648-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC