



AMD Ltd Zena Max Leptospira spp. PCR Detection Kit

CE

IVD



KD347540-100

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

The assay is an *in vitro* PCR test for the qualitative screening and identification of *Leptospira spp.* (including, but not limited to, *Leptospira interrogans*) DNA in human samples, such as whole blood and urine, is detected based on the hydrolysis probe detection method and is a highly sensitive qPCR kit.

For use in *in vitro* diagnostics.

Overview

Leptospira spp. are spiral-shaped bacteria (spirochetes) that have attracted significant attention because of their impact on human and animal health. First identified in the early 20th century, these bacteria are found across the globe, including in Africa, the Americas, Europe, and Asia. *Leptospira* primarily circulate among wildlife and domestic animals, particularly rodents, that shed the bacteria in their urine. Humans and other animals become infected through contact with contaminated water, soil, or urine, rather than via mosquito vectors. While many infections are mild or asymptomatic, leptospirosis can cause flu-like symptoms such as fever, headache, and muscle aches. In severe cases, it may lead to serious complications, including liver or kidney failure, haemorrhage, or meningitis, which can be life-threatening.

Principle of the Test

Detection of *Leptospira spp.* is essential for patient management and for the effective implementation of public health measures. Real-time PCR provides a reliable clinical laboratory tool for qualitatively detecting target nucleic acids present in patient samples.

During PCR amplification, forward and reverse primers hybridise to specific regions of the *Leptospira* DNA. A fluorogenic probe is included in the reaction mixture; this probe is an oligonucleotide labelled with a FAM 5' reporter dye and a downstream 3' quencher. As the target DNA strand is synthesised, the probe is cleaved by the 5'→3' exonuclease activity of Taq polymerase, separating the reporter from the quencher. This separation produces a measurable increase in FAM channel fluorescence, which is recorded by the detector as amplification progresses. If *Leptospira* DNA is not present in the sample, no gene-specific fluorescence signal will be generated.

The assay contains pairs of forward and reverse primers, as well as probes labelled with HEX 5' fluorescent reporter dyes and 3' quenchers. An internal positive control is included to assess the quality of the extracted DNA and identify any PCR inhibitors that may be present. These assays are multiplexed in a ready-to-use PCR master mix that employs hot-start technology to minimise non-specific amplification and maximise sensitivity. The mixture also contains uracil-DNA glycosylase (UDG), which prevents contamination from previously amplified PCR products.

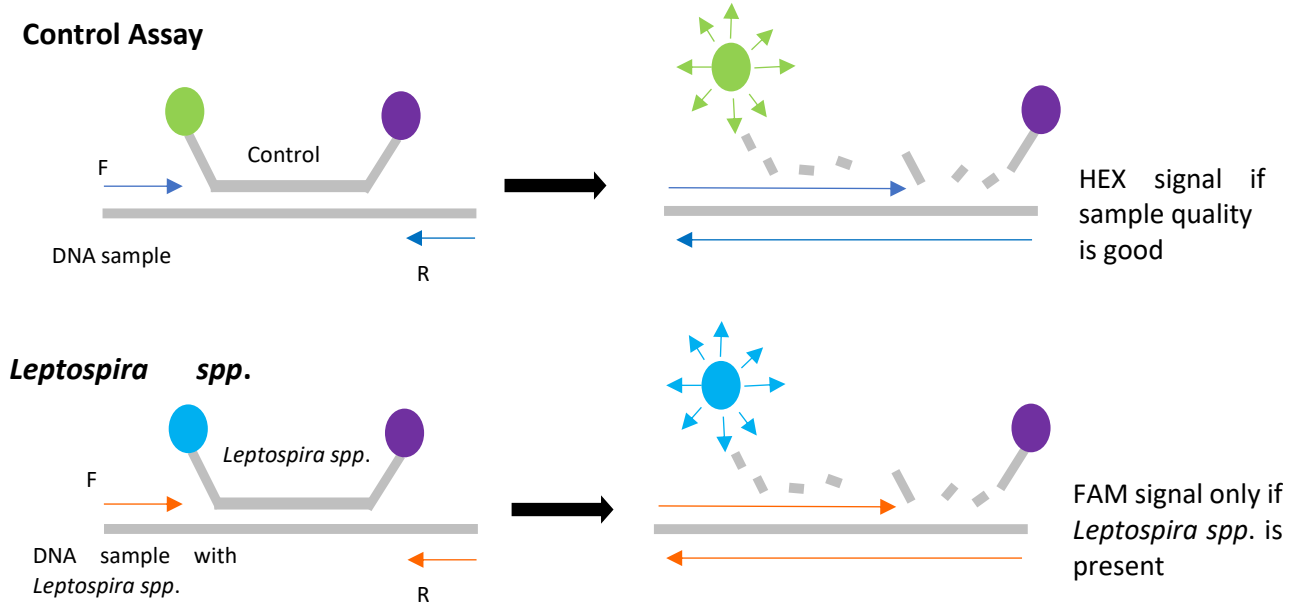


Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of *Leptospira* spp. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The *Leptospira* spp. The assay will produce a FAM signal if *Leptospira* spp. is present; 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 Contents

Item	
<i>Leptospira</i> spp. qPCR Master Mix	2 x 1 ml
<i>Leptospira</i> 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 be 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 (not provided)

DNA Extraction: AMD manufactures the LUCO AMD NA Extraction Kit, which can be used for extracting DNA from the samples. Other leading brands of IVD DNA extraction kits 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: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes. It is also compatible with both low and high ROX instruments.

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 accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD Zena Max *Leptospira* spp. qPCR Kit.

Instrument compatibility

AMD *Leptospira* spp. qPCR detection kit is compatible with the most common Real Time qPCR equipment with the capability of detecting FAM and HEX fluorescent dyes such as Biorad CFX96, Applied Biosystems 7500 Fast, QuantStudio 3,5,7, StepOne Plus, Agilent Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Sample collection, Storage and Transport

Whole blood can be taken into tubes containing EDTA for plasma or no anticoagulant for serum and stored for a maximum of 6 hours at 2-8°C before sample preparation. To prepare the sample for testing, separate the plasma from whole blood or the serum from clotted blood by centrifugation for 20 minutes at 800-1600 x g, then transfer to sterile polypropylene tubes. Plasma or serum can be stored for up to 6 days at 2-8°C. Aliquot and store the samples at -20°C or -80°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°C and no longer than 6 hours following collection.

Assay Procedure

Sample preparation

For optimal results use the LUCO NA Extraction Kit to isolate DNA from the samples. The resulting nucleic acid will be a mixture of DNA and RNA. 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.

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
<i>Leptospira spp.</i> qPCR M. Mix	20 µl
DNA Sample/control	5 µl

3. Add the DNA samples and standards 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 min
Stage 1: Step 2	95°C	2 min
40 cycles		
Stage 2: Step 1	95°C	10 sec
Stage 2: Step 2*	58°C	30 sec

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

6. When setting up the sample information in the qPCR software, enter the remark of the *Leptospira spp.* controls and define them as control in order to automatically obtain a result and qualitative of the amount of *Leptospira spp.* which may be present in the sample.
7. 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 identification of *Leptospira spp.* in a sample Table one as a quick reference guide:

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



HEX (Internal control)	FAM (<i>Leptospira. spp.</i>)	Interpretation
Positive (Ct<34)	No Cq	Sample Negative for <i>Leptospira spp.</i>
Positive	Positive (Ct<38)	Sample Positive for <i>Leptospira spp.</i>
No Cq	Positive (Ct<38)	Sample Positive for <i>Leptospira spp.</i>
No Cq	No Cq	Result inconclusive

Table 1. Interpretation of the results obtained from the Zena Max *Leptospira spp.* qPCR Kit.

Technical Specifications

Quality: All AMD kits are manufactured under high quality standardization methods with unique precision and sensitive technology when compared to the most of famous and approved diagnostic commercial *Leptospira spp.* PCR kits.

Sensitivity: AMD *Leptospira spp.* kit is a very sensitive kit, reaching up to 10 copies per rxn “rxn volume 25µl” under our validation methods and devices.

Specificity: AMD *Leptospira spp.* kit is 100% specific for *Leptospira spp.*

Data Analysis

Similar to other real-time PCR software, 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 each qPCR run with the gene expression module.

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 *Leptospira spp.* PCR Kit.

References

Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. *Leptospira spp.*. The Lancet infectious diseases. 2002 Sep 1;2(9):519-29.



Rossi, S.L., Ross, T.M. and Evans, J.D., 2010. *Leptospira spp.*. *Clinics in laboratory medicine*, 30(1), pp.47-65.

Petersen, L.R., Marfin, A.A. and Gubler, D.J., 2003. *Leptospira spp.*. *Jama*, 290(4), pp.524-528.

Contact


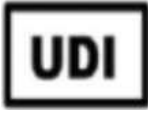




If you have any queries, comments or complaints please refer to our website at:

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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: 506105998LEPTCH UDI-DI: (01)05061059980854 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD347540-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC