AMD Ltd Zena Max Yersinia Pestis qPCR Detection Kit

CE IVD

REF **KD347530-100**

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

info@am-diagnostics.co.uk
+44(0)115 969 9934

AMD Ltd
BioCity Nottingham
Pennyfoot Street
Nottingham
NG1 1GF
United Kingdom

EC REP **Advena Ltd. Tower Business Centre, 2nd Flr., Tower Street, Swatar, BKR 4013 Malta**

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Intended Use

The assay is an *in vitro* qPCR assay for the qualitative determination of *Yersinia pestis* DNA in human samples and animal samples. These samples include blood plasma, sputum, or faeces. Detection based on the hydrolysis probe method for *Yersinia pestis* with a highly sensitive one step qPCR kit.
For use in *in vitro* diagnostics.

Overview

Yersinia pestis is a gram-negative bacterium. The bacteria can cause plague; septicaemic, bubonic, and pneumonic. The bacterium is zoonotic as it may reside in mammals, most notably rats. However, it is fleas that tends to be the main vectors of the bacterium. In addition to zoonotic vectors the bacterium can infect surfaces and become airborne in mucus droplets.
If diagnosed early, and with the correct course of antibiotics, patients can recover. However, this requires fast and accurate identification of the bacterium to tailor the treatment.

Principle of the Test

This kit is designed for the detection of *Yersinia pestis* by the Polymerase Chain Reaction (PCR) method. *Yersinia pestis* detection is based on the amplification of a specific conserved DNA sequence for the gens *caf1* and *pla* and measuring the amplification using fluorescently-labelled probes. *Y. pestis* presence is indicated by an increase in the fluorescence on the FAM fluorophore. 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. This 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.

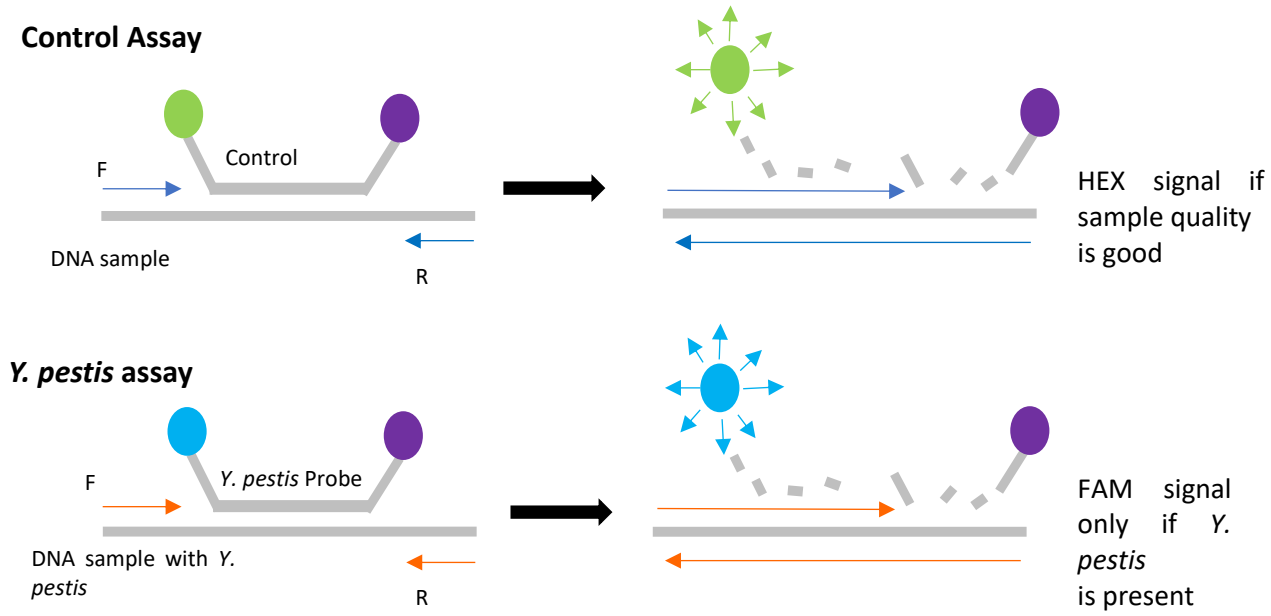

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of *Y. pestis.* The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. The *Y. pestis* assay will produce a FAM signal, if *Y. pestis* is present, however if it is not 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

Product	
***Y. pestis* qPCR M. Mix**	2 x 1 ml
Y. pestis Positive control	1 X 0.1 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 (not provided)

DNA Extraction: For optimal results use AMD DNA Extraction Kit to elute the DNA from the sample. Other leading kits or in-house methods are acceptable for use with this diagnostic kit, providing that it has been validated prior to use on patient samples.

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 *Yersinia pestis* qPCR Kit.

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^{0}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^{0}C. 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 leading brands of IVD DNA extraction kits with this diagnostic kit. 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 on a cool block or ice using the table below, ensuring to include duplicate reactions for all samples and controls.

Product	Volume X1
Y. Pestis PCR M. Mix	20 µl
DNA Sample/control	5 µl*

***Quantity per sample. Add directly to the PCR tubes/plate.**

3. Add the DNA samples and standards to the PCR tubes/plate. Add 5 µl 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	2mins
Stage 1: Step 2	95°C	2mins
40 Cycles		
Stage 2: Step 1	95°C	10secs
Stage 2: Step 2	58°C	30secs

*Data collection step in FAM (diagnostic assays) 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 which indicates the presence or absence of *Yersinia pestis* DNA. The results should be interpreted as follows:

- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for *Yersinia pestis* and the amount present in the original sample can be calculated using the equation below.
- If there is a HEX signal but no FAM signal, the sample is **negative** for *Yersinia pestis*.

- 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 no signal in either channel, the result is **inconclusive**.

Result		Interpretation
HEX	**FAM**	
Positive	Positive (Ct<38)	Positive for *Yersinia pestis*
No Cq	Positive (Ct<38)	Positive for *Yersinia pestis*
Positive (Ct<34)	No Cq	Negative for *Yersinia pestis*
No Cq	No Cq	Inconclusive

Table 1. Interpretation of the results obtained from the Zena Max *Yersinia pestis* qPCR Kit.

Technical Specifications

Quality: All AMD kits manufactured under high quality standardization methods and unique precision and sensitive technology study compared by most of famous and approved diagnostic commercial *Yersinia pestis* assays.

Sensitivity: The Zena Max *Yersinia pestis* qPCR kit is very sensitive kit reaches up to 2.4 copies/µl (assuming a 25 µl reaction).

Specificity: AMD *Yersinia pestis* kit is very specific under our validation methods and devices.

Product Limitations

The 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.

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
	Name and address of EU Representative	Advena Ltd Tower Business Centre, 2nd Floor, Tower Street, Swatar BKR 4013 Malta
	UDI-DI number for the product given	Basic: 506105998YPESFY UDI-DI: (01)05061059980434 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD347530-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC