AMD Ltd Zena Max *Helicobacter pylori* qPCR Detection Kit

CE IVD

REF **KD347533-100**

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 reaction assay for the qualitative determination of ***Helicobacter pylori*** (H. pylori) infection in a human sample such as fresh or frozen stool samples based on Taqman detection method with highly sensitive one step qPCR kit.

For *in vitro* diagnostic use.

Overview

Helicobacter Pylori is a gram-negative spiral bacterium belonging to the *Helicobacteracea* family, that is found to reside in 50% of persons worldwide[1]. It is responsible for causing gastric inflammation in virtually all infected persons, triggered primarily by the attachment of bacteria to epithelial cells[2] and, although acute *H. pylori* infection mainly passes asymptomatically, infection has been directly linked to several conditions, mainly peptic ulcer disease and no ulcer dyspepsia[3]. In addition to these conditions, nearly all Mucosa Associated Lymphoid Tissue (MALT) lymphoma patients are *H. pylori* positive, which increases the risk for development of gastric MALT lymphoma[4].

Principle of the Test

The Zena Max H. pylori PCR Detection Kit is designed for the qualitative detection of H. pylori by the real-time Polymerase Chain Reaction (PCR) method, which amplifies specific conserved DNA sequences, and fluorophore-labelled probes for the detection of amplified DNA. This assay contains two probes labelled with either the 5' FAM™ or 5' HEX™ reporter dye and a 3' quencher. The FAM™ labelled probe is specific for a conserved sequence within H. pylori DNA, whereas the HEX™ labelled probe is specific for a control gene present in the IC transcript. Two sets of forward and reverse primers are provided, annealing either side of each target gene. The bound probes are cleaved by the 5' to 3' exonuclease activity of Taq polymerase, releasing the fluorescent reporter from the quencher and substantially increasing the fluorescent signal.
The point at which the fluorescence becomes detectable above the background, the quantification cycle (Cq), is proportional to the amount of target present in the sample. The lower the Cq, the greater the amount of target present. If, however, H. pylori DNA is not present, a FAM signal will not be produced. The ready-to-use Master Mix (M. 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.
***This *in vitro* diagnostic kit provides qualitative detection.**

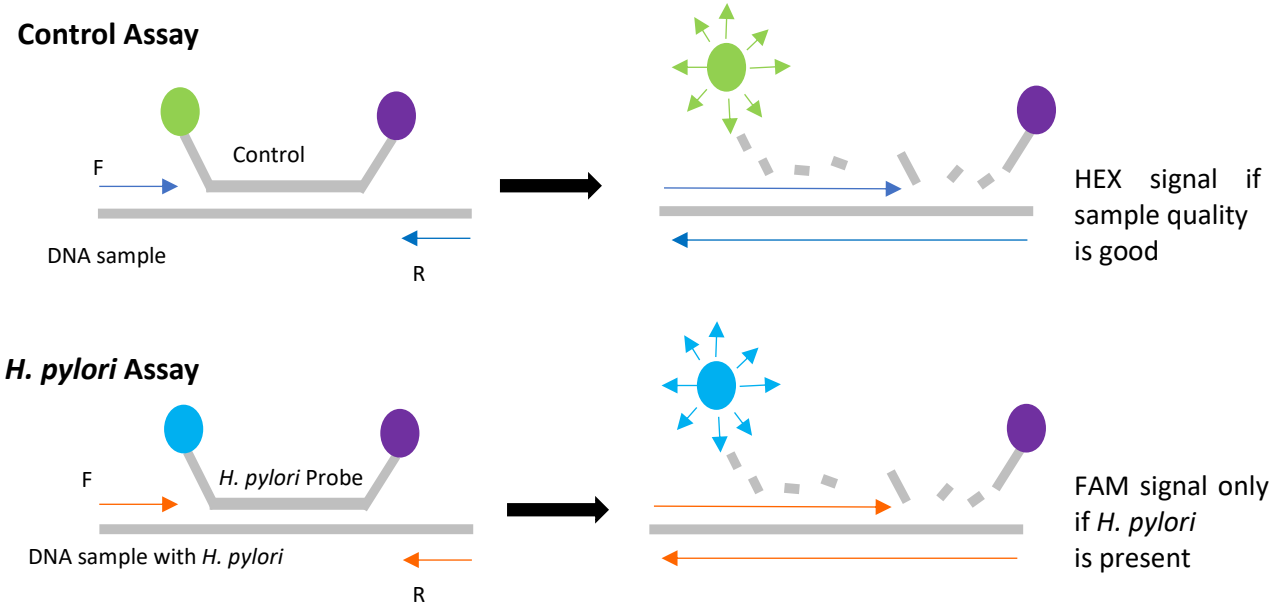

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of H. pylori DNA. The control assay in the master mix will produce a HEX signal if the DNA quality is acceptable. If a positive sample is tested, the *H. pylori* assay will produce a FAM fluorescent signal to indicate the pathogen DNA is present in the patient sample. Due to assay competition, the HEX signal may be reduced or absent when other signals are strong. If a negative sample is tested, only the HEX signal will be detected.

Provided Materials

Kit Contents

Item	
H. pylori qPCR M. Mix	2 x 1 ml
H. pylori 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 (not provided)

DNA Extraction: Other leading brands of 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: This kit should be used with qPCR systems which can detect FAM and HEX fluorescent dyes.

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 *H. pylori* qPCR Kit.

Instrument compatibility

AMD *Helicobacter pylori* 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, Aglient Mx3000, 3005P, Rotorgene Q, Cepheid Smartcycler, Analytik Jena qTower and Roche Lightcycler 480, 96.

Assay Procedure

Sample Collection

The sample for AMD *Helicobacter pylori* PCR Kit should be collected via samples from fresh or frozen stool samples. Please ensure that the sample is stored correctly and kept away from any contamination. 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.

Sample Transport

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, at a temperature of 2-8˚C, no longer than 6 hours following collection and by courier, if possible.

Sample Preparation

For optimal results use AMD DNA Extraction Kit to elute the DNA from fresh or frozen stool samples. 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 *Helicobacter pylori* qPCR M. Mix	20 µl
DNA Sample	5 µl

3. Add the DNA samples and the *Helicobacter pylori* control to the PCR tubes/plate. Include a No Template Control (NTC) by adding 5 µl nuclease free water to replace DNA.
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 secs
Stage 2: Step 2	58°C	30 secs

*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 and indicates the presence of absence of *H. pylori* in a sample. The results should be interpreted as follows, using Table one as a quick reference guide:

7. 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 (FAM) signal is strong.
8. If there is a signal in the **FAM** channel, with or without a HEX signal, the sample is **positive** for *H. pylori*.
9. If there is a **HEX** signal but no **FAM** signal, the sample is **negative** for H. pylori.
10. If there is no signal in either channel, the result is **inconclusive**.

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

Table 1. Interpretation of the results obtained from the Zena Max *H. pylori* qPCR Kit.

Performance Characteristics

Quality: All AMD kits are manufactured under high quality standard methods and unique precision, comparable with other approved diagnostic commercial *H. pylori* assays.
Sensitivity: AMD *Helicobacter pylori* kit is a very sensitive kit, reaching 100 copies / rxn "rxn volume 25µl" under our validation methods and devices.
Specificity: AMD *Helicobacter pylori* kit is very specific to *H. Pylori* under our validation methods and devices.

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.
False Negative results may arise from several factors including improper specimens as "collection, storage, and transportation methods" or degradation of the DNA during sample shipping and storage, the presence of inhibitors or other types of interfering substances.
Occasionally mutations may arise in the region of the genome targeted by the primers and probes of this assay, leading to reduction in performance or failure of the assay. Assay design and efficacy is 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.

References

1. Del Giudice G, Covacci A, Telford JL, Montecucco C, Rappuoli R. The design of vaccines against Helicobacter pylori and their development. Annual review of immunology. 2001;19:523.
2. Suerbaum S, Michetti P. Helicobacter pylori infection. New England Journal of Medicine. 2002 Oct 10;347(15):1175-86.
3. Crowe SE. Helicobacter pylori infection. New England Journal of Medicine. 2019 Mar 21;380(12):1158-65.

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
	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: 506105998HPYLDP UDI-DI: (01)05061059980403 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-15 to -25 degrees Celsius
	Catalogue number	KD347533-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC