



AMD Ltd Zena Max Hepatitis E Virus (HEV) PCR Kit - Quantitative



IVD



KD91241-100

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 reaction for the quantitative identification of HEV RNA in human samples such as EDTA plasma. It is based on hydrolysis probe detection and is a highly sensitive one-step RT-qPCR kit.

For *in vitro* diagnostic use.

Overview

Human Hepatitis E Virus (HEV) is a single-stranded ribonucleic acid (RNA) genome. It is the only member of the genus *Herpesvirus* in the family *Herpesviridae*. The virus has at least 4 different types: genotypes 1, 2, 3 and 4. Genotypes 1 and 2 have been found only in humans. Genotype 3 and 4 viruses circulate in several animals (including pigs, wild boars, and deer) without causing any disease, and occasionally infect humans.

The virus is shed in the stools of infected persons and enters the human body through the intestine. It is transmitted mainly through contaminated drinking water. Usually, the infection is self-limiting and resolves within 2–6 weeks. Occasionally a serious disease, known as fulminant hepatitis (acute liver failure) develops, and a proportion of people with this disease can die.

Principles of the Test

This kit detects the presence of HEV using one-step RT-qPCR (both reactions in the same tube) by first reverse transcribing the genomic RNA to cDNA, followed by amplification of the assay target and detection by the hydrolysis probe method of qPCR. The assay is designed to detect a highly conserved region of the HEV genome and consists of a forward primer, a reverse primer and a probe labelled with a 5' FAM™ reporter dye and a 3' quencher. As the new target cDNA strand is synthesised, the tightly bound probe is cleaved by the 5' to 3' exonuclease activity of Taq polymerase which releases the fluorescent reporter from the quencher and substantially increases the fluorescent signal. The point at which the fluorescence becomes detectable above the background, the quantification cycle (C_q), is proportional to the amount of target present in the sample. The lower the C_q, the greater the amount of target present.

If, however HEV is not present, a FAM signal will not be detected above background levels.

An internal positive control assay is provided in order to assess the quality of the isolated RNA and the effect of any PCR inhibitors that may be present. This assay contains two primers and a HEX labelled probe, designed to a highly conserved region of human RNA and a positive signal indicates that the RNA quality in the sample is acceptable for diagnostic testing, see Figure 1. These assays are both incorporated into a ready-to-use PCR master mix which utilises hot start technology, thus minimising non-specific reactions and ensuring maximum sensitivity.

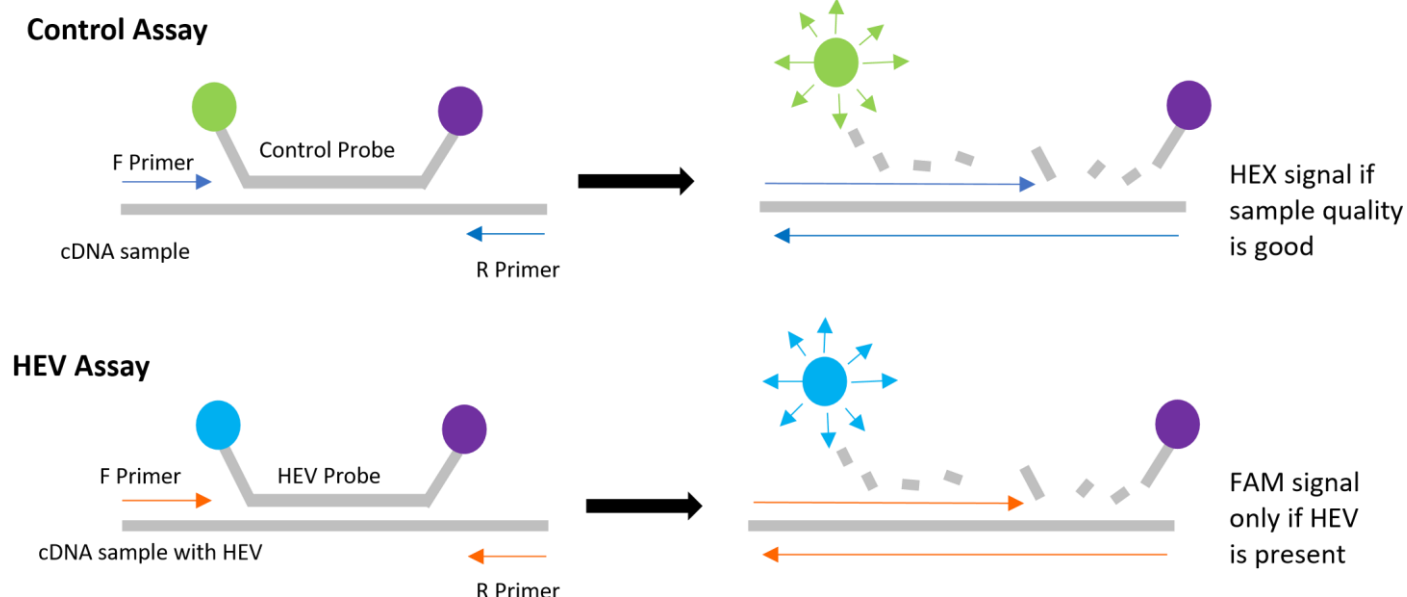


Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of HEV. The control assay in the master mix will produce a HEX signal if the cDNA quality is acceptable. The HEV assay will produce a FAM signal, if HEV is present, however, if it is not present, no FAM signal 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

Items	
HEV one-step RT-qPCR M. Mix	2 x 1 ml
HEV Standard 1 (1.0×10^8 copies/ml)	1 x 0.05 ml
HEV Standard 2 (1.0×10^7 copies/ml)	1 x 0.05 ml
HEV Standard 3 (1.0×10^6 copies/ml)	1 x 0.05 ml
HEV Standard 4 (1.0×10^5 copies/ml)	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 freeze-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 reagent is thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (but not provided)

RNA Isolation: AMD recommends using the Luco-Viral NA Extraction Kit to isolate RNA from the samples. 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: Use nuclease-free PCR consumables appropriate to the qPCR instrument. It is also recommended that the correct PPE, including gloves, is used throughout.

Other Laboratory Equipment: Vortex, microcentrifuge, micropipettes and tips, microfuge tubes and 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). Treat all samples as potentially infected. 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. Before using the AMD Zena Max HEV qPCR Kit, please familiarise yourself with this product manual and your qPCR instrument.

Instrument compatibility

AMD HEV 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 either be collected in tubes containing EDTA 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 by centrifugation for 20 minutes at 800-1600 x g, then transfer to sterile polypropylene tubes. Plasma 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 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, at a temperature of 2-8°C, no longer than 6 hours following collection and by courier, if possible.

Assay Procedure

Sample Preparation

For optimal results use the Luco-Viral NA Extraction Kit to isolate RNA 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 RNA. Store the RNA 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 RNA.



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. If preferred, make a mix of assay and reverse enzyme mix and aliquot 20µl into the PCR tubes/plate.

Product	Volume x1
HEV M. Mix	20µl
RNA sample/standard	5µl*

*Add directly to the PCR tubes/plate.

3. Add the RNA samples and the four HEV controls to the PCR tubes/plate. Also, 5µl nuclease-free water should be added in place of the RNA 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	55°C	15 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 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.

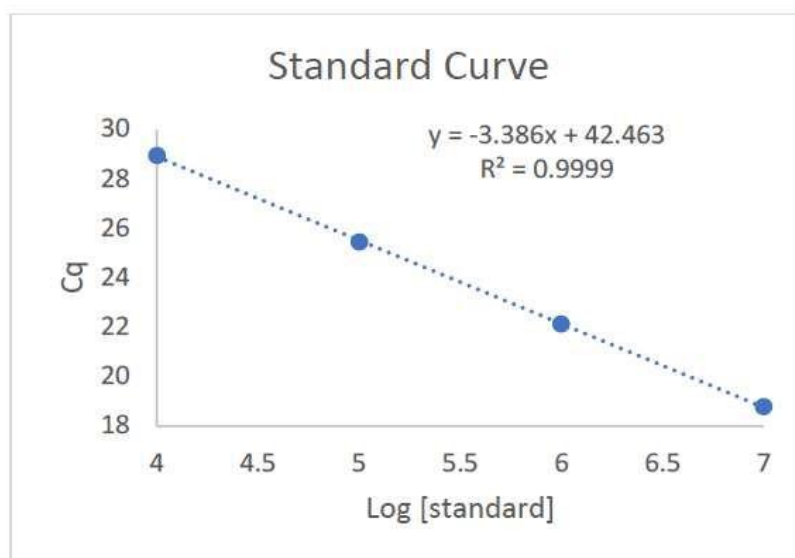


Figure 2: An example of the standard curve using the quantification cycles of the four HEV standards. This equation can be used to quantify the amount of HEV if it is present in the qPCR reactions.

Interpretation of Results

This is a quantitative assay and the results are scored based on whether a Cq value is present or not. Results should be interpreted as follows, using Table 1 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 HEV.
- If there is a HEX signal but no FAM signal, the sample is **negative** for HEV.
- If there is no signal in either channel, the result is **inconclusive**.

Results		Interpretation
HEX	FAM	
Positive	Positive (Ct<38)	Positive for HEV
Positive (Ct<34)	No Cq	Negative for HEV
No Cq	Positive (Ct<38)	Positive for HEV
No Cq	No Cq	Inconclusive



Quality Control

Quality: All AMD kits are manufactured under high-quality standard methods and unique precision, comparable with other leading commercial HEV diagnostic kits.

Sensitivity: The Zena Max HEV qPCR kit is highly sensitive and able to detect a minimum of 10 copies per 25µl reaction under our validation methods and devices.

Specificity: This AMD HEV kit is up to 100% specific for HEV all relevant HEV genotypes 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 assay, leading to a 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.

References

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Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, Dalton HR. Hepatitis e. The Lancet. 2012 Jun 30;379(9835):2477-88.

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Contact

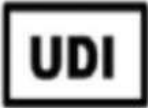
If you have any queries, comments or complaints please refer to our website at:

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: 506105998HEVH8 UDI-DI: (01)05061059980540 UDI-PI: See label
	Minimum and maximum storage temperatures for this product	-18 to -25 degrees Celsius
	Catalogue number	KD91241-100
	Number of tests/reactions in this pack	100
	CE-IVD certified	According to Directive 98/79/EC