Advanced Molecular Diagnostics Zena Max SARS-CoV-2 qPCR Detection Kit

CE IVD

Product Information:

Catalogue Number KD145904N-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

1. This assay is an *in-vitro* PCR test for the qualitative identification of **new Corona virus 2019 COVID-19** RNA in human samples such as nasopharyngeal and oropharyngeal swab. It is based on the hydrolysis probe detection method and is a highly sensitive one-step RT-qPCR kit.

For *in vitro* diagnostic use.

Overview

Corona virus 2019 COVID-19 is the virus responsible for respiratory disease caused by a novel (new) coronavirus that was first detected in Wuhan City, Hubei Province, China . The virus is contagious and can be spread from human to human primarily through the exchange of mucus droplets that are expelled through sneezes or coughs.
The virus that causes the disease coronavirus disease 2019 COVID-19
The SARS-CoV-2 (2019-nCoV) is a severe acute respiratory syndrome coronavirus outbreak is an important reminder that the global community must strengthen national and international programs for detection and response to future disease outbreaks.

Principles of the test

This kit detects the presence of **COVID-19** using one-step RT-qPCR (both reactions in the same tube) by first reverse transcribing the genomic RNA target to cDNA, followed by amplification of the assay target and detection by the hydrolysis probe method of qPCR. The assay consists of a forward primer, a reverse primer and a probe labelled with the 5' FAM™ and ROX reporter dye with 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 (Cq), is proportional to the amount of target present in

the sample. The primers and probe sets target N Gene which had previously been used in the identification of the SARS coronavirus, however, there is no cross-reactivity with this or any other coronavirus sequenced thus far. The SARS CoV-2 (2019-nCoV) genomes are designed for the in vitro detection of SARS COV-2 genomes. The lower the Cq, the greater the amount of target present. If, however **Corona virus 2019 COVID-19** is not present, a FAM signal signal will not be produced. 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. 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.

This *in vitro* diagnostic kit provides qualitative detection.

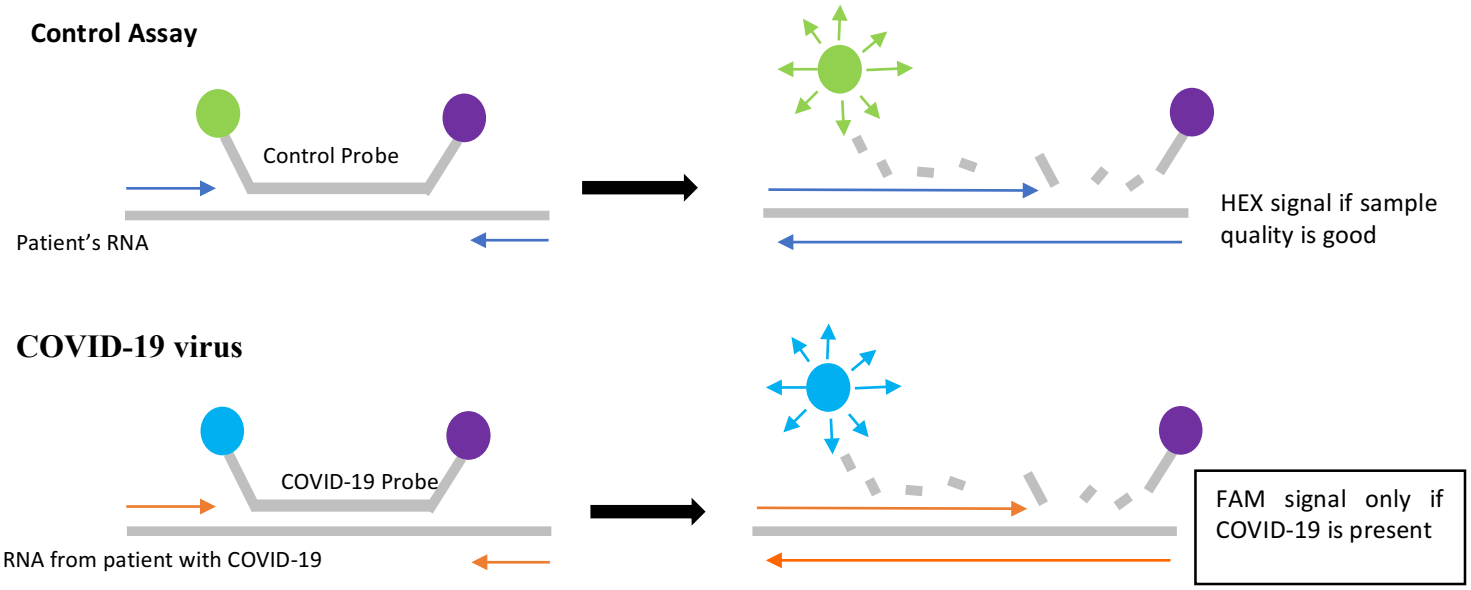

Figure 1. The principle of qPCR with hydrolysis probe detection for identifying the presence of COVID-19. The control assay in the master mix will produce a HEX signal if the RNA quality is acceptable. The COVID-19 assay will produce a FAM signal, if COVID-19 virus is present, however if it is not present, 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

1 x 1.9 ml COVID-19 FAM and Human RNase P HEX multiplex qPCR master mix

1 x 0.1ml RT Enzyme Mix

1 x 0.05 ml COVID-19 virus control

1 x 1 ml PCR Grade Water (nuclease free)

Reagent Storage and Handling

The kits should be transported and stored at temperatures between -30°C and -15°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 are thoroughly thawed, mixed and pulse centrifuged before use.

Materials and Equipment Required (but not provided)

RNA Extraction: AMD recommends using the Luco-Viral NA Extraction Kit to extract DNA from the samples. Other leading kits, such as the QIAamp MinElute Virus Spin Kit (Qiagen) 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.

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 precisely follow the instructions in this manual, to ensure accurate results. Please familiarise yourself with this product manual and your qPCR instrument before using the AMD Zena Max COVID-19 qPCR Kit.

Assay Procedure

Sample Collection

The sample for use with this kit is according to WHO recommendation the upper respiratory specimens like nasopharyngeal and oropharyngeal swab or wash in ambulatory patients or the lower respiratory specimens like sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. Please ensure that the sample is stored correctly and kept away from any contamination.

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. Quantify the RNA using a spectrophotometer and dilute to 3-5ng/µl in nuclease free water or nuclease free TE buffer pH8.0. 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 using the table below.

Product	Volume x1	Volume x 10
Master mix including multiplex assay	19µl	190µl
Reverse transcriptase	1µl	10µl
RNA sample/Control	5µl	5µl*

*Add directly to the PCR tubes/plate.

3. Add the RNA samples and the COVID-19 control to the PCR tubes/plate. Also add 5µl nuclease free water 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	5 min
40 Cycles		
Stage 2: Step 1	95°C	10 sec
Stage 2: Step 2*	55°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.

Interpretation of Results

This is a qualitative assay and indicates the presence or absence of COVID-19. The results should be interpreted as follows, using 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 signal is strong.
- If there is a signal in the FAM (green) channel, with or without a HEX signal, the sample is **positive** for COVID-19 virus.
- If there is a HEX signal but no FAM signal, the sample is **negative** for COVID-19 virus.
- If there is no signal in either channels, the result is **inconclusive**.

Result		
HEX	**FAM**	**Interpretation**
Positive	Positive	Positive for SARS-CoV-2
No Cq	Positive	Positive for SARS-CoV-2
Positive	No Cq	Negative for SARS-CoV-2
No Cq	No Cq	Inconclusive

Table 1. Interpretation of the results obtained from the Zena Max COVID-19 virus qPCR Kit.

Quality Control

Quality: All AMD kits are manufactured under high quality standard methods and unique precision, comparable with other leading commercial COVID-19 virus diagnostic kits.

Sensitivity: The Zena Max COVID-19 qPCR kit is highly sensitive, able to detect a minimum of 1 copies per reaction under our validation methods and devices.

Specificity: This AMD COVID-19 kit is up to 100% specific for the COVID-19 N gene under our validation methods and devices.

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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 region of the genome targeted by the primers and probes of this assay, leading to reduction in performance or failure of the assay. The 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.

Contact

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

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