

Primer Design Ltd

R01064

Middle East respiratory syndrome Coronavirus (MERS-CoV):

Kit version: 1

Target region:

Open Reading Frame 1ab (ORF1ab)

Open Reading Frame 5/E (ORF5/E)

genesig[®] Advanced Kit

150 tests

GENESIG

Kits by Primerdesign

Specificity of primers and probes last reviewed on:
07 March 2022

For general laboratory and research use only

Introduction to MERS-CoV

Middle East Respiratory Syndrome coronavirus (MERS-CoV) is the cause of Middle East Respiratory Syndrome (MERS), also known as camel flu. MERS is a viral respiratory illness that was first reported in Saudi Arabia in 2012, causing periodic outbreaks primarily in the Middle East and, to a lesser extent, in other parts of the world. It is characterized by severe respiratory symptoms, including fever, cough, and shortness of breath. The virus is believed to have originated in camels and can be transmitted to humans through close contact with infected animals or by human-to-human transmission in healthcare settings.

MERS-CoV gained international attention due to its potential for rapid spread and its relatively high mortality rate, particularly among individuals with preexisting health conditions. Though it has not reached pandemic proportions like some other coronaviruses, such as SARS and COVID-19, MERS remains a concern for global health authorities, necessitating ongoing surveillance and research to better understand and control the disease.

Specificity

The genesig[®] Advanced Kit for Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) is designed for the in vitro quantification of MERS genomes. The kit is designed to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of the last review.

This kit comprises two assays, each targeting different genes within the MERS-CoV genome. In the event that a strain of MERS-CoV exhibits substantial sequence variation in one of these gene targets, potentially causing detection issues, the other assay should still detect the strain. This provides the kit with a greater resilience against new circulating variants.

The dynamics of genetic variation mean that new sequence information may become available after the most recent review. Primer Design Ltd periodically reviews the detection profiles of our kits and when required releases new versions.

If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

- 1x MERS_CoV_ORF1ab primer/probe mix (150 reactions, **BROWN**)
FAM labelled.
- 1x MERS_CoV_ORF5/E primer/probe mix (150 reactions, **BROWN**)
FAM labelled.
- 1x MERS_CoV_ORF1ab positive control template (**RED**)
- 1x MERS_CoV_ORF5/E positive control template (**RED**)
- 2x Internal extraction control primer/probe mix (150 reactions, **BROWN**)
VIC labelled as standard.
- 1x Internal extraction control RNA template (150 reactions, **BLUE**)
- 1x Endogenous control primer/probe mix (150 reactions, **BROWN**)
FAM labelled.
- 1x RNase/DNase free water (**WHITE**)
for resuspension of primer/probe mixes
- 3x Template preparation buffer (**YELLOW**)
for resuspension of internal control template, positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA Extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high quality RNA and DNA with minimal PCR inhibitors.

oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix

This kit is intended for use with oasig® lyophilised OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix

Pipettors and filter tips

Vortex and centrifuge

1.5 ml tubes

qPCR plates or tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA integrity (An internal PCR control is supplied to test for non-specific PCR inhibitors). Always run at least one negative control with the samples.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template. If running a positive control standard curve for a quantitative result, and an efficiency of between 90% to 110% is not achieved, then the run should be repeated with a freshly prepared standard curve.

Notices and disclaimers

This product is developed, designed, and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

PrecisionPLUS® is a trademark of Primer Design Ltd.

genesig® is a registered trademark of Primer Design Ltd.

oasig® is a trademark of Primer Design Ltd.

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. TaqMan® is a registered trademark of Roche Molecular Systems, Inc., The purchase of the Primer Design Ltd reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.

Principles of the test

Real-time PCR

MERS specific primer/probe mixes are provided, and these can be detected through the FAM channel.

The primer/probe mixes provided exploits the so-called TaqMan[®] principle. During PCR amplification, forward and reverse primers hybridize to the target cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains positive control templates. This can be used to generate standard curves of target copy number/Cq value. Alternatively, the positive controls can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target genes worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive controls do not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control wells.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal RNA extraction control

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate qPCR primer/probe mix is supplied with this kit to detect the exogenous RNA using qPCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the target cDNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

Endogenous control

To confirm extraction of a valid biological template, a primer/probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel, and it is NOT therefore possible to perform a multiplex with the target primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening.

This will ensure lyophilised primer probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. Resuspend the primer/probe mixes in the RNase/DNase free water supplied, according to the table below:

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in water	Volume
Pre-PCR pack	
MERS_CoV_ORF1ab primer/probe mix (BROWN)	165 µl
MERS_CoV_ORF5/E primer/probe mix (BROWN)	165 µl
Internal extraction control primer/probe mix (BROWN)	165 µl
Endogenous control primer/probe mix (BROWN)	165 µl

3. Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:

To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control RNA template (BLUE)	600 µl
Post-PCR heat-sealed foil	
MERS_CoV_ORF1ab Positive Control Template (RED) *	500 µl
MERS_CoV_ORF5/E Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. Add 4µl of the Internal extraction control RNA (**BLUE**) to each sample in RNA lysis/extraction buffer per sample.
2. Complete RNA extraction according to the manufacturer's protocols.

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. For each RNA sample prepare a reaction mix according to the table below: Include sufficient reactions for positive and negative controls.

Component	Volume
oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
MERS_CoV_ORF1ab or MERS_CoV_ORF5/E primer/probe mix (BROWN)	1 µl
Internal extraction control primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	3 µl
Final Volume	15 µl

2. For each RNA sample prepare an endogenous control reaction according to the table below (optional):

This control reaction will provide useful information regarding the quality of the biological sample.

Component	Volume
oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
Endogenous control primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	15 µl

3. Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.

4. Pipette 5µl of RNA template into each well, according to your experimental plate set up.

For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

5. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:

Component	Volume
oasig® OneStep or PrecisionPLUS® OneStep 2X RT-qPCR Master Mix	10 µl
MERS_CoV_ORF1ab or MERS_CoV_ORF5/E primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	15 µl

6. Preparation of standard curve dilution series.

- a. Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
- b. Pipette 10µl of Positive Control Template (**RED**) into tube 2
- c. Vortex thoroughly
- d. Change pipette tip and pipette 10µl from tube 2 into tube 3
- e. Vortex thoroughly

Repeat steps d and e to complete the dilution series.

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2×10^5 per µl
Tube 2	2×10^4 per µl
Tube 3	2×10^3 per µl
Tube 4	2×10^2 per µl
Tube 5	20 per µl
Tube 6	2 per µl

7. Pipette 5µl of standard template into each well for the standard curve according to your plate set-up

The final volume in each well is 20µl.

OneStep RT-qPCR Amplification Protocol

Amplification conditions using oasisig® OneStep or Precision®PLUS OneStep 2X RT-qPCR Master Mix.

	Step	Time	Temp
	Reverse Transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

* Fluorogenic data should be collected during this step through the FAM and VIC channels.

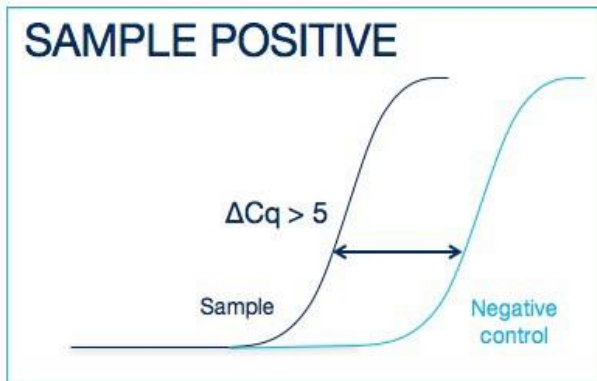
Interpretation of results

Target (FAM)	Internal control (VIC)	Positive control	Negative control	Interpretation
≤ 30	+ / -	+	-	POSITIVE QUANTITATIVE RESULT * calculate copy number
> 30	+	+	-	POSITIVE QUANTITATIVE RESULT * calculate copy number
> 30	-	+	-	POSITIVE QUALITATIVE RESULT * do not report copy number as this may be due to poor sample extraction
-	+	+	-	NEGATIVE RESULT
+ / -	+ / -	+	≤ 35	EXPERIMENT FAILED due to test contamination
+ / -	+ / -	+	> 35	**
-	-	+	-	SAMPLE PREPARATION FAILED
+ / -	+ / -	-	+ / -	EXPERIMENT FAILED

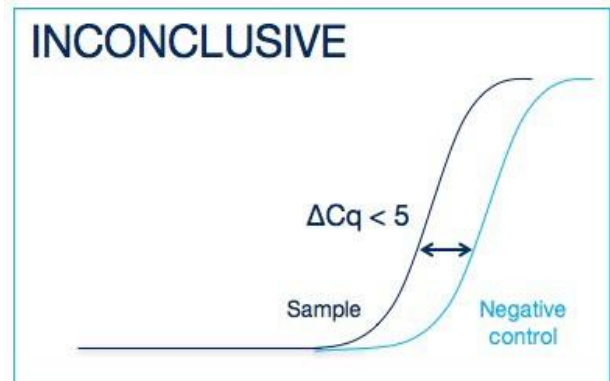
Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* A positive result for MERS-CoV is indicated by a positive result for one or both targets.

** Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28 ± 3 are within the normal range. When amplifying an target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.