Human Metapneumovirus

Nucleoprotein gene

Standard Kit

150 tests

For general laboratory and research use only

Introduction to Human Metapneumovirus

Human metapneumovirus (hMPV) was isolated for the first time in 2001 in the Netherlands by using the RAP-PCR technique for identification of unknown viruses growing in cultured cells. hMPV is a negative single-stranded RNA virus of the family Paramyxoviridae and is closely related to the avian metapneumovirus (AMPV) subgroup C. It may be the second most common cause (after the respiratory syncytial virus) of lower respiratory infection in young children.

Compared with respiratory syncytial virus, infection with human metapneumovirus tends to occur in slightly older children and to produce disease that is less severe. Co-infection with both viruses can occur, and is generally associated with worse disease. Human metapneumovirus accounts for approximately 10% of respiratory tract infections that are not related to previously known etiologic agents. The virus seems to be distributed worldwide and to have a seasonal distribution with its incidence comparable to that for the influenza viruses during winter. Serologic studies have shown that by the age of five, virtually all children have been exposed to the virus and reinfections appear to be common. Human metapneumovirus may cause mild respiratory tract infection however small children, elderly and immunocompromised individuals are at risk of severe disease and hospitalization.

Specificity

The Kit for Human Metapneumovirus (HMPV) genomes is designed for the in vitro quantification of HMPV genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the HMPV genome.
The primers and probe sequences in this kit have 100% homology with a broad range of HMPV sequences based on a comprehensive bioinformatics analysis.

The primers have 100% homology with all reference sequences in the NCBI database and therefore have a very broadest quantification profile. However, due to the inherent instability of RNA viral genomes, it is not possible guarantee quantification of all clinical isolates.

Kit Contents

- **HMPV specific primer/probe mix (150 reactions BROWN)**
  FAM labelled
- **HMPV positive control template (for Standard curve RED)**
- **HMPV RT primer mix (150 reactions GREEN)**
  Required for two step protocol only
- **RNAse/DNAse free water (WHITE)**
  for resuspension of primer/probe mixes
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

RNA extraction kit

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

oasig™ Lyophilised OneStep or Precision™ OneStepPLUS 2x qRT-PCR Mastermix

Contains complete one step qRT-PCR Mastermix

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC 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.

We do 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/DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNAse/DNAse free water.

Dynamic range of test

Under optimal PCR conditions HMPV detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

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 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 Applera 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

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Principles of the test

Real-time PCR

A HMPV specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the HMPV cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled 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 real-time PCR platforms.

One Step vs. Two step real-time PCR

When detecting/quantifying the presence of a target with an RNA genome recommend the use of a one step qRT-PCR protocol. One step qRT-PCR combines the reverse transcription and real-time PCR reaction in a simple closed tube protocol. This saves significant bench time but also reduces errors. The sensitivity of a one step protocol is also greater than a two step because the entire biological sample is available to the PCR without dilution. This kit will also work well with a two step approach (Precision™ nanoScript2 reverse transcription kit and PrecisionPLUS™ Mastermix) if required but the use of oasig™ OneStep or Precision™ OneStepPLUS Mastermix is the preferred method.

Positive control

For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of HMPV copy number / Cq value. Alternatively the positive control 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 HMPV gene 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 control does 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 well.

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.

Carry-over prevention using UNG (unsuitable for onestep procedure and optional for two step procedure)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. We recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step.

Reconstitution 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 and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Reconstitute the kit components 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	
HMPV primer/probe mix (BROWN)	165 µl
HMPV RT primer mix (GREEN)	165 µl

3. **Reconstitute the positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
HMPV 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.

One Step RT-PCR detection protocol

A one step approach combining the reverse transcription and amplification in a single closed tube is the preferred method. If, however, a two step approach is required see page 10.

For optimum performance and sensitivity.

All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the One Step 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 Precision™ OneStepPLUS 2x qRT-PCR Mastermix	10 µl
HMPV primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**

3. **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.

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

Component	Volume
oasig™ OneStep or Precision™ OneStepPLUS 2x qRT-PCR Mastermix	10 µl
HMPV primer/probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

5. Preparation of standard curve dilution series

1) Pipette 90µl of template preparation buffer into 5 tubes and label 2-6
2) Pipette 10µl of Positive Control Template (RED) into tube 2
3) Vortex thoroughly
4) Change pipette tip and pipette 10µl from tube 2 into tube 3
5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

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

6. 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.

One Step Amplification Protocol

Amplification conditions using oasig™ OneStep or Precision™ OneStepPLUS 2x qRT-PCR Mastermix.

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

* Fluorogenic data should be collected during this step through the FAM channel

Alternative two step reverse transcription/real-time PCR protocol

Reverse Transcription

1. **After reverse transcription, prepare a reaction mix according to the table below for each cDNA sample**

Component	Volume
PrecisionPLUS™ 2x qPCR Mastermix	10 µl
HMPV Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.**

3. **Prepare sample cDNA templates for each of your samples by diluting the RT reaction mix 1:5 in RNAse/DNAse free water.**

4. **Pipette 5µl of cDNA template into each well, according to your experimental plate setup.**
The final volume in each well is 20µl. For negative control wells use 5µl of RNAse/DNAse free water.

Alternative two step amplification protocol

Amplification conditions using PrecisionPLUS™ 2x qPCR Mastermix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37 °C
	Enzyme activation	2 mins	95 °C
Cycling x50	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

* Fluorogenic data should be collected during this step through the FAM channel
** Required if your Mastermix includes UNG to prevent PCR carryover contamination

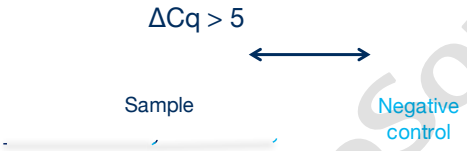
Interpretation of Results

Target	Positive control	Negative control	Interpretation
+	+	-	**POSITIVE QUANTITATIVE RESULT** calculate copy number
-	+	-	**NEGATIVE RESULT**
+ / -	+	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	+	> 35	*
+ / -	-	+ / -	**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

*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:

SAMPLE POSITIVE



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.

INCONCLUSIVE

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 smaple should be repeated.