ARGENE

Parechovirus r-gene®

Meningo-Encephalitis Multi Well System r-gene®
Real Time RT-PCR kit

Product code: 71-020

CE
IVD

1. Product Description

Intended use:

The Parechovirus r-gene® kit enables rapid detection of the Human Parechovirus (HPeV) using 5' nuclease technique, a real time analysis technology.

Parechoviruses have an estimated prevalence of 95% of the world population. They are responsible for infections that, although mostly asymptomatic, may still lead to severe diseases (eg encephalitis, meningitis, pneumonia).

The diagnosis is usually based on viral culture, potentially followed by a sero-neutralization. This technique is very sensitive for most HPeV is long and tedious.
Parechovirus r-gene® significantly improves the diagnosis of HPeV allowing early detection and improved sensitivity with all serotypes of the human Parechovirus.

Principle:

The amplification premix is optimised for amplification and detection of the human Parechovirus (reading at 530 nm) simultaneously with the internal control 1 (IC1) (reading at 560 nm).
This real-time duplex PCR is performed after extraction of nucleic acids in samples of CSF, respiratory specimens and faeces.

Amplified sequence :

- Parechovirus:
  Region 5'UTR.
  Fragment size amplified : 256 bp.

Additional reagents/instruments required:

Other products from the Multi Well System r-gene® range listed in Chapter « Related Products » can be used in combination with this product.

- Validated extraction platforms:
  - MagNAPure Compact
  - NucliSENS easyMAG®
- Validated Real-Time PCR amplification platforms:
  - LightCycler® 480
  - Applied Biosystems 7500 Fast, Step One®
  - Stratagene®/Versant® kPCR Molecular Systems AD/Agilent.
  - Rotor-gene®
  - Dx Real-Time System (Bio-Rad)

Number of reactions:

60 reactions assuming a pipetting volume of 15 µL.
Final reaction volume: 25 µL.

Content:

R20	Amplification premix Parechovirus and IC1 *Contains dNTPs, $MgCl_2$, amplification buffer, primers and probes for Parechovirus, internal control 1 (IC1), Taq Polymerase, passive reference ROX™.*	2 x 450 µL
W0	Water for extraction (molecular grade)	1 x 1.8 mL
PC20	Positive control	1 x 300 µL
IC1	Internal control 1	1 x 0.7 mL
RT	Reverse transcriptase (Concentrated)	1 x 15 µL

Package Insert : 1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib

Storage:

Store at -18°C/-22°C before and after first opening, protected from light until the expiration date printed on the label, in the room reserved for preparation of the premixes.

Store the positive control and the internal control before and after first opening at -18°C/-22°C in the same place as the extracted samples.

Controls:

- The negative extraction and amplification control (**IC1W0** à 530 nm)
This control consists of the internal control (IC1) added to water (**W0**). This mixture is extracted and amplified at the same time and using the same protocol as for the patient samples.
This control checks for contamination in the extraction and amplification.
Signal reading is 530 nm.

- Internal control 1 (**IC1W0** à 560 nm)
Internal control is added to IC1 from the lysis step:
- to the sample to be extracted.
It is the control for extraction + inhibition of the sample (**IC1sample**).
- to the water to extract (**W0**).
It is the control for extraction + reference inhibition (**IC1W0**).
⇒ The Comparison of CT values of both **IC2W0** and **IC2sample** controls at 560 nm is used to evaluate the efficacy of the extraction and detect the presence of any inhibitors.

- The positive control (PC20)
This control (PC20) contains a plasmid which is specifically recognised by the primers and probes for Parechovirus contained in the reagent mixture R20.
Systematically tested, it verifies the proper outcome of the amplification step.
Signal reading is 530 nm.

- The cellular control :

The cellular control checks the presence of cells in the samples. If Rhino&EV/Cc r-gene® (Argene product code : 71-042) of the Respiratory Multi Well System r-gene® range is tested, cellular control is included.

If not, the CELL Control r-gene® (Argene ref.: 71-106) kit must be used.

2. Warnings and Precautions

Amplification procedures require highly skilled techniques to avoid risk of sample contamination:

- Use separate working places for sample preparation and amplification reactions. Movement in the laboratory must be in one direction only from the reagent preparation area to the amplification area. Allocate a set of lab coats and pipettes to each area. Never introduce an amplified product in reagent and/or sample preparation areas.
- Used samples must be exclusively reserved for this analysis.
- Samples must be prepared under a biological safety hood.
- Tubes from different specimens and amplification premix must never be opened at the same time.
- Always perform preventive maintenance for workstations, for automated extraction, amplification, and centrifuge systems, according to the manufacturer's recommendations.
- Pipettes used to handle samples are reserved for this purpose only. These pipettes must be positive displacement pipettes or pipettes equipped with filter tips. All tips must be sterile.
- The pipettes used to aliquot reagents must be reserved only for this purpose. The necessary reagents for amplification are aliquoted in order to be used during one single experiment.
- Handle and dispose all specimens and materials as potentially infectious. After use: material, reagents and waste must be handled as potentially infectious.
- Do not use reagents after expiration date printed on the labels.
- Never pipet by mouth.
- Do not smoke, eat or drink in dedicated work areas.

3. Product Handling

3.1 Extraction

Instruments	Kit	Sample volume	Sample type	Protocol	Elution volume
MagNAPure Compact (Roche Diagnostics)	MagNAPure Compact Nucleic acid Isolation Kit I (Ref. : 03 730 964 001)	200 µL of sample + 10 µL **IC1**	CSF, respiratory specimens, faeces	Total_NA_plasma_100_400	50 µL
		400 µL of sample + 10 µL **IC1**	Respiratory specimens, faeces		100 µL
NucliSENS® easyMAG® * (bioMérieux)	NucliSENS® easyMAG® EasyMag Reagents	200 µL of sample + 10 µL **IC1**	CSF, respiratory specimens, faeces	Specific B	50 µL
		400 µL of sample + 10 µL **IC1**	Respiratory specimens, faeces		100 µL

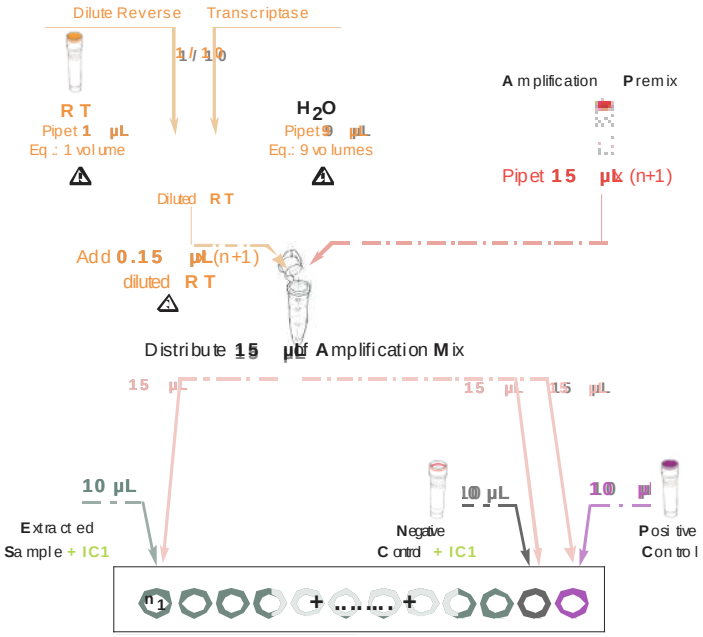
* For certain respiratory samples and in case of extraction with the NucliSENS® easyMAG® apparatus, a pre-treatment of the samples with proteinase K is required. In this case, add 10 µL of proteinase K at 20 mg/mL for 200 µL of sample and allow to incubate for 15 min. at 56°C.

3.2 Amplification

Note: To simplify this protocol, the device containing the amplification reaction mix will be referred to as a "well".

3.2.1 Amplification preparation

- Plan **n** wells *(**n**= number of samples + Positive Control + Negative Control).*



⚠ It is recommended to use pipettes adapted to the volumes required in the experiment.

Example:

For 7 samples to be analysed in one run:

***n** = 7 + 2 = 9*
Pipet (n+1) x 0.15 µL of RT diluted = 1.5 µL
Add (n+1) x 15 µL of amplification premix = 150 µL
Homogenize the reaction mixture
Distribute 15 µL of this mix in each well.

- Centrifuge for 15 sec. (depending on the instrument).
- Place the microplate in the real time PCR instrument and run the program as described in the table below.

3.2.2 Amplification program

Steps		Time	Temperature	Cycles	Fluorescence Acquisition
Reverse Transcription		5 min.	50°C	1	-
Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing	40 sec.	60°C		530 + 560 nm
	Elongation	25 sec.	72°C		-

Note1: The temperature increases and decreases are set by default, which means at 100% or at their maxima.

Note 2: On LihtCycler® 480, two optical systems exist: only "System II" is compatible with the use of the kit. The "System II" features in its software a colour compensation to be activated.

Note 3: On Applied Biosystems 7500 Fast, only, select «ROX» as «PASSIVE REFERENCE» when programming.

Note 4: On Applied Biosystems StepOne®, only select «NONE» in «PASSIVE REFERENCE» when programming.

Note 5: On Rotor-Gene®, calibrate the signal by clicking on «GAIN OPTIMISATION».

Note 6: On Stratagene® or Versant® kPCR Molecular System AD or Agilent select «NONE» in «REFERENCE DYE» when programming.

Detailed programming guides for each real time PCR platform are available on request.

4. Results

4.1 Validation of results

The test is only valid if all the following conditions are fulfilled. If this is not the case, all samples and controls must be tested again.

1st condition: The negative control should not give a detectable signal at 530 nm.
2nd condition: The positive control must give a signal below **32 cycles** at 530 nm.
3thrd condition : The extraction control + inhibition reference **IC1W0** should give a signal (CT) less than or equal to **32 cycles** at 560 nm.

4.2 Interpretation of results

- There is a 530 nm CT for all positive samples.
- The absence of CT at 530 nm corresponds to a negative, inhibited or poorly extracted sample.
- A strongly positive sample may generate inhibition. This has no effect on the analysis.

1st Step: Reading at 530 nm **Parechovirus**	2nd Step: Reading at 560 nm **IC1sample, IC1W0**	**Interpretation**
CT calculated	No need to validate	For POSITIVE sample **Parechovirus**
No CT calculated	CT [IC1sample]≤ CT [IC1W0] + 3 cycles	For NEGATIVE sample **Parechovirus**
	CT [IC1sample]> CT [IC1W0] + 3 cycles	Result NOT VALIDATED *(inhibited or poorly extracted sample -> Re do sample test)*

Note: The quality of the sample taken for the pathogen search can be verified using the cellular control (CELL Control r-gene® - ref.: 71-106, also contained in the reagents: Rhino&EV/CC r-gene® - ref.: 71-042).

IMPORTANT NOTE:
It is absolutely necessary to compare results obtained with this Argene kit with other diagnostic investigation methods in order to define patient infectious status.

5. Performance of the assay

The performance of the Parechovirus r-gene® kit validated in the technical documentation of the CE branding will be available in the next version of the data sheet.

6. References

Specific real-time PCR platform programming and analysing guidelines are available.

7. Related Products

Respiratory Multi Well System r-gene®

- *Influenza* A/B r-gene® Argene ref.: 71-040
- RSV/hMPV r-gene® Argene ref.: 71-041
- Rhino&EV/Cc r-gene® Argene ref.: 71-042
- AdV/hBoV r-gene® Argene ref.: 71-043
- Chla/Myco pneumo r-gene® Argene ref.: 71-044
- HCoV/HPIV r-gene® Argene ref.: 71-045

Meningo-Encephalitis Multi Well System r-gene®

- Parechovirus r-gene® Argene ref.: 71-020

Controls Range

- DICO Ampli r-gene® Argene ref.: 71-100
- DICO Extra r-gene® Argene ref.: 71-101
- RICO Extra r-gene® Argene ref.: 71-105
- CELL Control r-gene® Argene ref.: 71-106

8. Index of symbols

Symbol	Meaning
	Catalogue number
	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Protect from light
	Keep dry
	Identification of notified body

BIOMÉRIEUX
bioMérieux SA
Chemin de l'Orme
69280 Marcy-l'Etoile - France
RCS LYON 673 620 399
Tel. 33 (0)4 78 87 20 00
Fax 33 (0)4 78 87 20 90
www.biomerieux.com