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For research use only

Viral Pneumonia
REAL-TIME PCR Detection Kit

REF **R3-P428-S3/6EU**

Package S

General information

Intended use:
Viral Pneumonia REAL-TIME PCR Detection Kit is intended for detection of the most common causative agents of acute viral respiratory infections, Influenza A (including A (H1N1)) and Influenza B by Real-Time PCR method. **Viral Pneumonia REAL-TIME PCR Detection Kit** can be used in scientific research practice.

Method:
Real-Time Reverse Transcription PCR, qualitative analysis.

Samples:
Swabs and washings from nasal cavity and oropharynx.

RNA extraction:
The DNA-Technology's **PREP-NA PLUS DNA/RNA Extraction Kit** are recommended.

Features:
Multiplex analysis – simultaneous detection of multiple targets in the one tube.
PCR-mix contains an internal control (IC). IC is intended for PCR quality assurance.
Positive control plasmid (C+) supplied with the kit is intended for specific PCR assessment.
We also recommend including in assay the negative control (C-) which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for **Viral Pneumonia REAL-TIME PCR Detection Kit** is available on "DNA-Technology" made DTlite[1] and DTprime[2] REAL-TIME Thermal Cyclers; the latest version of the software is available for download at https://www.dna-technology.com/software.

Time of analysis (including sample preparation procedure):
from 2.5 hours.

Number of tests:
12 (including one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
Reverse Transcription Kit			
1. RT-buffer	Colorless transparent liquid	100 µL	1 tube
2. RT-random primers and dNTP's	Colorless transparent liquid	50 µL	1 tube
3. Reverse transcriptase	Colorless transparent viscous liquid	25 µL	1 tube
PCR-amplification Kit			
1. Paraffin sealed PCR-mix:	Colorless or blue transparent liquid under white wax layer		
Strip 1		20 µL in each	12 8-tubes strips
Strip 2		20 µL in each	12 8-tubes strips
2. PCR-buffer	Colorless transparent liquid	500 µL	4 tubes
3. Taq-polymerase	Colorless transparent viscous liquid	50 µL	2 tubes
4. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	4 tubes
5. Positive control	Colorless transparent liquid	300 µL	1 tube
Associated accessories:			
Strip's caps			24 8-caps

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.
[2] supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.

Strip content, color codes and detection channels

№ of tube	Detection channel		Color labeling of the buffer
	Fam	Hex	
Strip 1			
1	Influenza A virus	IC	Blue
2	Influenza B virus	IC	Colorless
3	Influenza A virus (H1N1)-like pandemic A/California/4/2009	IC	
4	Human metapneumovirus	IC	
5	Human respiratory syncytial virus	IC	
6	Human rhinovirus	IC	
7	Human adenovirus	IC	
8	Human bocavirus	IC	
Strip 2			
1	Human parainfluenza virus 1	IC	Colorless
2	Human parainfluenza virus 2	IC	Blue
3	Human parainfluenza virus 3	IC	Colorless
4	Human parainfluenza virus 4	IC	
5	Human coronavirus OC43	IC	
6	Human coronavirus 229E	IC	
7	Human coronavirus HKU1	IC	
8	Human coronavirus NL63	IC	

Procedure

1 Nucleic acids extraction

⚠ Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of NA extraction simultaneously with the NA extraction from clinical samples.

Physiological saline solution can be used as a negative control sample in volumes as indicated in the instructions for use of extraction kits or negative control sample that is included in the corresponding extraction kit.

1.1 Perform sample preparation procedure according to the instruction to NA-extraction kit (DNA Technology's **PREP-NA PLUS DNA/RNA Extraction Kit**). Use RNase and DNase free pipette tips only.

⚠ After drying the precipitate should be dissolved in 100 µL of dilution buffer. DNA-IC and RNA-IC are not used. Resulting RNA preparation must be used immediately for reverse transcription. The RNA preparation can not be stored.

2 Reverse transcription

2.1 Mark the required number of 0.5 mL tubes (with lock, in case you do not have thermostat with clamping cover) according to the number of samples to be analyzed and 1 tube for negative control (C-).

2.2 Thaw content of RT-buffer and RT-random primers and dNTP's tubes from **Reverse Transcription Kit** at room temperature (from 18 °C to 25 °C), then vortex thoroughly. Then spin briefly for 3-5 s.

2.3 Prepare RT-mix. Add to the one tube:
- 4.0 x (N+1) µl of RT-buffer,
- 2.0 x (N+1) µl of RT-random primers and dNTP's,
- 1.0 x (N+1) µl of reverse transcriptase,

N – is a quantity of the samples to be tested taking to account "C-".

Example: to test 5 samples, mark 6 tubes. Prepare RT-mix for 7 (6+1) tubes. Mix 28 µL of RT-buffer, 14 µL of primers and 7.0 µL of reverse transcriptase.

⚠ Reverse transcriptase should be kept out of freezer for as short time as possible.

2.4 Vortex the tube with RT-mix. Then spin briefly for 3-5 s.

2.5 Add 7.0 µL of the RT-mix to all marked tubes.

2.6 Add 33 µL of the corresponding NA samples to the tubes with RT-mix. Do not add NA sample to the "C-" tube.

2.7 Add 33 µL of "C-" sample, which passed the whole NA extraction procedure to the "C-" tube.

⚠ Use filter tips to prevent contamination.

2.8 Vortex the tubes and spin down the drops for 3-5 s.

2.9 Place tubes in thermostat and incubate at 40 °C for 30 min, then heat up to 95 °C and leave for 5 min.

⚠ Use "DNA-Technology" Gnom Programmable thermostat or similar thermostats with clamping cover.

2.10 Spin the tubes at RCF(g) 16000 for 30 s to collect drops.

2.11 Add 60 µL of the dilution buffer from **PREP-NA PLUS DNA/RNA Extraction Kit** to the obtained preparation of cDNA. Vortex and spin the tubes for 3-5 s to spin down the drops.

The cDNA preparation is ready for PCR.

3 PCR amplification

The reagents and tubes should be kept away from direct sun light!

Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

3.1 Mark the required number of strips (strip 1 and strip 2) with paraffin sealed PCR-mix: 2 strips for each sample to be tested, 2 strips for positive control (C+) and 2 strips for negative control (C-).

Example: to test 2 samples, mark 4 strips for analyzed samples, 2 strips for "C-" and 2 strips for "C+". The resulting number of strips – 8.

Sample 1	Strip 1, Strip 2
Sample 2	Strip 1, Strip 2
"C-"	Strip 1, Strip 2
"C+"	Strip 1, Strip 2

3.2 Thaw the PCR-buffer at room temperature, then vortex tubes with PCR-buffer and Taq-polymerase thoroughly and spin down drops for 3-5 s.

Taq-polymerase should be got out from freezer just prior to use.

3.3 Prepare mixture of PCR-buffer and Taq-polymerase. Add to the one tube:

- 160 x N+10 µL of PCR-buffer,
- 8 x N+0.5 µL of Taq-polymerase,

N – is a quantity of the samples to be tested taking to account "C-" and "C+".

Example: for simultaneous testing of 2 samples, 1 "C-" and 1"C+" in one PCR run, mix 650 µL of PCR-buffer and 32.5 µL of Taq-polymerase (calculate final volume for 4 tubes).

3.4 Vortex the tube thoroughly and spin down the drops for 3-5 s.

3.5 Add 10 µL of mixture of PCR-buffer and Taq-polymerase into the each tube of strips. Avoid paraffin layer break.

3.6 Add one drop (~20 µL) of mineral oil into each tube. Close strips.

Open the strip, add cDNA sample (or control sample), then close the strip before proceeding to the next strip to prevent contamination. Use filter tips. Close strips tightly.

3.7 Add 5.0 µL of cDNA sample into corresponding strip. Use filter tips. Do not add cDNA into the "C-", "C+" strips.

3.8 Add 5.0 µL of "C-" which passed the whole NA extraction procedures into "C-" strips. Add 5.0 µL of "C+" into "C+" strips. Avoid paraffin layer break.

3.9 Spin strips for 1-3 s to collect drops.

3.10 Set the strips to the thermal cycler.

3.11 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 3.10) and run PCR.

4 Data collection and data analysis

Registration of the PCR results is held in automatic mode. Interpretation of the PCR results should be performed according to the Table 1.

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 1. Interpretation of PCR results

Detection channel		Result	Result interpretation
Fam	**Hex**		
Analyzed samples			
Cp is specified (for one or several tubes)	Is not considered	+	NA of one or several pathogens is detected
Cp is not specified (for one or several tubes)	Cp is specified (for the same tubes which are negative on the Fam channel)	-	NA of pathogens is not detected
Cp is not specified (for one or several tubes)	Cp is not specified (for the same tubes which are negative on the Fam channel)	invalid	Unreliable result[5]
Positive control sample			
Cp is specified (for all tubes)	Cp is specified (for all tubes)	+	Positive result The results are valid
Negative control sample			
Cp is not specified (for all tubes)	Cp is specified (for all tubes)	-	Negative result The results are valid

Storage, shipping and handling requirements

All kit components, except strips with paraffin sealed PCR-mix and positive control, must be stored at the temperatures from minus 18 °C to minus 22 °C during the storage period.

Multiple freezing of PCR-buffer and mineral oil is allowed.

The strips with paraffin sealed PCR-mix should be stored in a dark place from 2 °C to 8 °C during the storage period.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions.

Transportation of the kit, except the Taq-polymerase, PCR-buffer, mineral oil and Reverse transcription Kit, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the Taq-polymerase, PCR-buffer, mineral oil and Reverse transcription Kit in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life – 12 months if all the conditions of transportation, storage and operation are met.

Contact our customer service department regarding quality issues with the kit:

8 800 200-75-15 (toll-free call for Russia)

+7 (495) 640-16-93 (chargeable call for CIS and foreign countries).

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Key to symbols

	Temperature limit		Consult instructions for use		Catalogue number
	Use-by date		Manufacturer		Batch code
	Date of manufacture		Contains sufficient for <n> tests		Keep away from sunlight
	Caution		Non-sterile		Do not reuse

[5] - repeat PCR amplification or NA extraction for the given sample, performed sequentially.