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For research use only

Influenza A virus, Influenza B virus Multiplex Real-Time PCR Detection Kit

REF **R3-P431-23/4EU**
R3-P431-S3/4EU

Package S

General information

Intended use:
Influenza A virus, Influenza B virus Multiplex REAL-TIME PCR Detection Kit is intended for detection of Influenza A and B viruses RNA in the human and animal biological samples.
Influenza A virus, Influenza B virus Multiplex REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
Multiplex Real-Time with Reverse Transcription (RT-PCR), qualitative analysis.

Samples:
Smears and washings from nasal cavity and oropharyngeal cavity, trachea, bronchopulmonary lavage, biopsy samples.

RNA extraction:
The DNA-Technology's **PREP-NA DNA/RNA Extraction Kit** is recommended (see user manuals supplied with the **PREP-NA** kit).

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.

Devices:
The automatic analysis for **Influenza A virus, Influenza B virus Multiplex 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 (excluding sample preparation procedure):
from 2.5 hours.

Number of tests:
48 (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 transparent liquid under white wax layer	20 µL in each	48 tubes or 6 8-tubes strips
2. PCR-buffer	Colorless transparent liquid	500 µL	1 tube
3. Taq-polymerase	Colorless transparent viscous liquid	25 µL	1 tube
4. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
5. Positive control	Colorless transparent liquid	75 µL	1 tube
Associated accessories:			
Strip's caps[3]			6 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.

[3] - for detection kit packaged in strips REF R3-P431-S3/4EU

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
Influenza A virus	IC	Influenza B virus	-	-

Procedure

1 RNA extraction

⚠ Independently of DNA/RNA extraction kit used, a negative control sample should go through all stages of RNA extraction simultaneously with the RNA 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 RNA-extraction kit (DNA Technology's **PREP-NA DNA/RNA Extraction Kit**). Use RNase and DNase free pipette tips only.

⚠ After drying the precipitate should be dissolved in 50 µL of Dilution buffer. DNA-IC and RNA-IC are not used.
If it is needed, the resulting RNA preparation can be stored at temperatures from minus 18 °C to minus 22 °C for no longer than a week with a single defrosting before reverse transcription.

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:
- 2.0 x (N+1) µl of RT-buffer,
- 1.0 x (N+1) µl of RT-random primers and dNTP's,
- 0.5 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 14 µL of RT-buffer, 7.0 µL of primers and 3.5 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 3.5 µL of the RT-mix to all marked tubes.

2.6 Add 16.5 µL of the corresponding RNA samples to the tubes with RT-mix. Do not add RNA sample to the "C-" tube.

2.7 Add 16.5 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.

The resulting cDNA preparation is ready for the PCR amplification.

3 PCR amplification

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

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

3.1 Mark the required number of the tubes with paraffin sealed PCR-mix considering samples, negative control (C-) and positive control (C+).

Example: to test 2 samples, mark 4 tubes (one for each sample, one for "C-" and one for "C+").

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 Taq-polymerase solution of PCR-buffer and Taq-polymerase. Add to the one tube:
- 10 x (N+1) µL of PCR-buffer,
- 0.5 x (N+1) µL of Taq-polymerase,

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

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

⚠ Taq-polymerase solution must be prepared immediately prior to use and should be used within two hours after preparation.

3.5 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

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

⚠ Open the tube, add cDNA sample (or control sample), then close the tube before proceeding to the next sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next strip to prevent contamination.Use filter tips. Close tubes/strips tightly.

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

3.8 Add 5.0 µL of negative control sample (C-) which passed whole RNA extraction procedures and reverse transcription procedures into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

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

3.10 Set the tubes/strips to the thermal cycler.

3.11 Launch the operating software for DT instrument[4]. Add corresponding test[5], 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.

Table 1. Interpretation of PCR results

Detection channel			**Result**	**Interpretation**
Fam	**Hex**	**Rox**		
Analyzed samples				
Cp is not specified	Cp is specified	Cp is not specified	-	RNA of Influenza A and B viruses is not detected
Cp is specified	Is not considered	Cp is not specified	+	RNA of Influenza A virus is detected
Cp is not specified	Is not considered	Cp is specified	+	RNA of Influenza B virus is detected
Cp is specified	Is not considered	Cp is specified	+	RNA of Influenza A and Influenza B viruses is detected
Cp is not specified	Cp is not specified	Cp is not specified	n/a	Unreliable result[6]
Positive control sample				
Cp is specified	Is not considered	Cp is specified	+	Positive result The results are valid
Negative control sample				
Cp is not specified	Cp is specified	Cp is not specified	-	Negative result The results are valid

[4] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[5] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.
[6] - repeating of PCR or RNA extraction and Reverse transcription with PCR for the given sample is required. Is performed sequantially.

Storage, shipping and handling requirements

All kit components, except tubes (or 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 tubes (or strips) with Paraffin sealed PCR-mix should be stored in a dark place from 2 °C to 8 °C during the storage period.

Excessive temperature and light can be detrimental to product performance.

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 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 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 – 9 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

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