Customer service department:
Phone: +7 (495) 640-16-93,
Phone/Fax: +7 (495) 640-17-71.
E-mail: hotline@dna-technology.ru,
www.dna-technology.ru

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

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.

Method:

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

Samples:

Swabs and washings from nasal cavity, oropharyngeal cavity, trachea, bronchopulmonary lavage, biopsy samples.

RNA extraction:

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

Features:

Multiplex analysis gives the opportunity of several cDNA targets detection in the same tube.

PCR-Mix contains an internal control (IC). IC is intended for PCR quality control.

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 Influenza A virus, Influenza B virus Multiplex REAL-TIME PCR Detection Kit is available on "DNA-Technology" made DTlite[1], DTprime[2] and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.7.5.23; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

Overall time needed to perform the analysis (excluding sample preparation procedure):

from 2.5 hours.

The number of tests:

48

Kit contents:

Reagent	Quantity	
Reverse transcription reagents		
• Primers RT-RANDOM+dNTP`s	50 µL	1 tube
• RT-buffer	100 µL	1 tube
• Reverse transcriptase	25 µL	1 tube
PCR-amplification reagents		
• Paraffin sealed PCR-mix	20 µL	48 tubes or 6 8-tube strips
• Taq-polymerase	25 µL	1 tube
• PCR-buffer	500 µL	1 tube
• Mineral oil	1,0 mL	1 tube
• Positive control	75 µL	1 tube
Associated accessories:		
• Strip's caps[3]		6 8-caps strips

Dye label detection channels

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

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments.
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments.
[3] - in the case of stripped tubes use.

Procedure

1 RNA extraction

1.1 Mark the required number of the 1.5 mL plastic tubes considering samples and negative control ("C-").

1.2 Perform the RNA extraction procedure according to user manual supplied with the PREP-NA extraction kit.

Use RNase and DNase free pipette tips only.

The precipitate should be dissolved in 50 µL of the Dilution Buffer after drying. DNA-IC and RNA-IC are not used.

⚠ The resulting RNA preparation mustn't be stored. It must be used immediately for reverse transcription.

2 Reverse transcription (RT)

2.1 Mark the required number of 0.5 mL tubes: 1 tube for each test samples, 1 tube for negative control "C-".

2.2 Thaw the content of the tubes "RT buffer", "Primers RT-RANDOM + dNTP's" at the room temperature (from 18 °C to 25 °C). Vortex the tubes thoroughly, then spin briefly for 3-5 sec.

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 Primers RT-RANDOM + 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. If you need to test 5 samples, mark 6 tubes. Prepare RT mix for 7 tubes. Mix 14 µL of RT buffer, 7.0 µL of Primers RT-RANDOM + dNTP's and 3.5 µL of Reverse transcriptase.

⚠ Hold the Reverse transcriptase at room temperature as short time as possible. The overheating is detrimental to its performance.

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

2.5 Add 3.5 µL of RT mix into each marked tube. Close tubes tightly.

2.6 Add 16.5 µL of the extracted RNA sample into corresponding tube. Open the tube, add RNA sample, then close the tube before proceeding to the next RNA sample to prevent contamination. Use separate filter tip for each sample. Do not add the extracted RNA sample into the "C-" tube.

2.7 Add 16.5 µL of the "C-" which passed whole RNA extraction procedure into corresponding tube.

2.8 Vortex the tube thoroughly. Then spin briefly for 3-5 sec.

2.9 Place the tubes in a thermostat and incubate at 40 ° C for 30 minutes, then warm up at 95 ° C for 5 minutes.

⚠ It is recommended to use programmable thermostats with clamp lid (for example, DNA-Technology made "Gnom").

2.10 Centrifuge the tubes at 13 000 rpm for 30 seconds.

The resulting cDNA preparation is ready for the PCR amplification.

3 PCR amplification

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

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

3.2 Thaw the PCR buffer at room temperature. Vortex the PCR buffer and Taq-polymerase thoroughly for 3-5 sec, then spin briefly for 1-3 sec.

⚠ Taq-polymerase should be got out from the freezer immediately prior to use.

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

- 10.0 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-", "C+".

3.4 Vortex the tube thoroughly. Then spin briefly for 3-5 sec.

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

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

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

3.7 Add 5.0 µL of the cDNA sample into corresponding PCR-tube. Open the tube, add cDNA sample then close the tube before proceeding to the next cDNA sample to prevent contamination. 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 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 Vortex tubes for 1–3 seconds to collect drops.

3.10 Set the tubes to the real-time PCR thermal cycler.

3.11 Launch the RealTime_PCR application in "Device handling" mode. Upload "InflAB_en.ini" file before the first run. Add test in subsequent runs. Specify the number and identificator of samples. Define position of tubes in software interface according to position they were set in thermal unit (p. 3.10). Run PCR.

4. Data collection and data analysis

Registration and interpretation of the PCR results are held in automatic mode. PCR results interpretation should be carried out in accordance with Table 1.

Shipping, storage and handling requirements

All kit components, except tubes (or strips) with paraffin-sealed PCR-mix and positive control sample ("C+"), must be stored at the temperature between minus 18 °C and minus 22 °C during the storage period.

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

Tubes (or strips) with paraffin-sealed PCR-mix and positive control sample ("C+"), must be stored at the temperature between 2 °C and 8 °C and out of light during the storage period.

Transportation of kit's components can be held by all types of roofed transport at the temperatures corresponding to the storage conditions of individual reagents, included in the kit.

⚠ Tubes (or strips) with paraffin-sealed PCR-mix and positive control sample ("C+") must be transported at the temperature between 0 °C and 24 °C for not more than 72 hours.

⚠ Tubes with RT buffer, Primers RT-RANDOM+dNTP`s, Reverse transcriptase, PCR buffer, Taq-polymerase and mineral oil must be transported on ice (in a box with freezable gel-type packets) for not more than 72 hours.

Shelf-life – 9 months from the date of Quality Control Department approval in compliance with all transportation, storage and operation conditions.

Contact our customer service department regarding issues of quality Influenza A virus, Influenza B virus Multiplex REAL-TIME PCR Detection Kit:

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E-mail: hotline@dna-technology.ru, www.dna-technology.ru

Address: 117587, Moscow, Varshavskoye sh., 125g building 6, DNA Technology.

Table 1. Results interpretations

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[1]
Positive control sample				
Cp is specified	Is not considered	Cp is specified	+	Positive result
Negative control sample				
Cp is not specified	Cp is specified	Cp is not specified	-	Negative result

[1] - Repeating of PCR or RNA extraction with PCR for the given sample is required. Is performed sequantially.