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For research use only

C.pneumoniae, M.pneumoniae
Multiplex REAL-TIME PCR Detection Kit

Package S

REF **R1-P430-23/4EU**
R1-P430-S3/4EU

General information

Intended use:
C.pneumoniae, M.pneumoniae Multiplex REAL-TIME PCR Detection Kit is intended for of *Chlamydia (Chlamydophila) pneumoniae* and *Mycoplasma pneumoniae* DNA by the Real-Time PCR method.
C.pneumoniae, M.pneumoniae Multiplex REAL-TIME PCR Detection Kit can be used in scientific research practice.

Method:
Multiplex Real-Time PCR, qualitative analysis.

Samples:
Phlegm, blood, pleural fluid, aspirates, bronchopulmonary lavage, biopsy samples, smears and washings from nasal cavity and nasopharyngeal cavity.

DNA extraction:
The DNA-Technology's **PREP-RAPID**, **PREP-GS and PREP-NA** extraction kits are recommended (see instruction for use supplied with the DNA extraction kits).

Features:
Multiplex analysis gives the opportunity of several DNA targets detection in the same tube.
PCR-mix contains an internal control (IC). IC is intended for PCR quality assurance.
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 **C.pneumoniae, M.pneumoniae 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 1.5 hours.

The number of tests:
48 (including one positive control and one negative control in each run).

Kit contents:

Reagent	Organoleptic parameters	Quantity	
1. Paraffin sealed PCR-mix	Colorless transparent liquid under white wax layer	20 µL in each	6 8-tubes strips or 48 tubes
2. Taq-polymerase solution	Colorless transparent liquid	500 µL	1 tube
3. Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	1 tube
4. 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 R1-P430-S3/4EU.

Dye label detection channels

Fam	Hex	Rox	Cy5	Cy5.5
C. pneumoniae	IC	*M. pneumoniae*	-	-

Procedure

1 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!

1.1 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 2 tubes for samples, one for "C-", one for "C+". The resulting number of tubes is 4.

1.2 Vortex the Taq-polymerase solution thoroughly (3-5 s), then spin briefly (1-3 s).

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

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

1.5 Vortex the tubes with samples, "C-" and "C+" for 3-5 s and spin down drops for 1-3 s.

1. In case of using **PREP-GS Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute at room temperature (from 18 °C to 25 °C) to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 s in a vortex mixer.

Relative centrifugal force (RCF or g) depends on rotation frequency and rotor radius (Annex A). To establish if your centrifuge meets the requirements apply to the exploitation manual for centrifuge.

2. Open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA 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.

1.6 Add 5.0 µL of the DNA sample into corresponding tubes. Avoid paraffin layer break. Do not add DNA into the "C-", "C+" tubes.

1.7 Add 5.0 µL of negative control sample (C-), which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

1.8 Spin tubes/strips for 1–3 s to collect drops.

1.9 Set the tubes/strips to real-time PCR thermal cycler.

1.10 Launch the operating software for DT instrument[1]. Add corresponding test[2], 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 1.9) and run PCR.

2 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.

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[2] 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	Interpretation
Fam	Hex	Rox		
Analyzed samples				
Cp is specified	Is not considered	Cp is not specified	+	DNA of *C. pneumoniae* is detected
Cp is not specified	Is not considered	Cp is specified	+	DNA of *M. pneumoniae* is detected
Cp is specified	Is not considered	Cp is specified	+	DNA of *C. pneumoniae* and DNA of *M. pneumoniae* are detected
Cp is not specified	Cp is specified	Cp is not specified	-	DNA of pathogens is not 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 The results are valid
Negative control sample				
Cp is not specified	Cp is specified	Cp is not specified	-	Negative result The results are valid

Storage, shipping and handling requirements

All kit components should be stored at the temperatures from 2 °C to 8 °C during the storage period.

Paraffin-sealed PCR-mix should be stored at the temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit has to be transported in thermoboxes 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.

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)

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

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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

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

Annex A

Nomogram and formula for calculation of relative centrifugal force (RCF) in the speed of rotation (RPM) depending of the rotor diameter