GeneProof
Molecular diagnostics for your routine

GeneProof Flu Multiplex PCR Kit

CE IVD

In vitro diagnostic medical device
The kit has been manufactured according to EC Directive 98/79/EC as an in vitro diagnostic medical device and it has been designed for professional use in specialized clinical and research laboratories.

KIT CONTENT

IC supplied in a separate tube
Nucleic acid extraction and PCR inhibition control

REF	FLU/GP/025 25 rxn	FLU/GP/050 50 rxn	FLU/GP/100 100 rxn
Master Mix Flu	1x375 µl	2x375 µl	4x375 µl
Positive Control Flu	1x200 µl	1x200 µl	2x200 µl
Internal Control RNA IC	1x250 µl	2x250 µl	4x250 µl

STORAGE AND TRANSPORTATION CONDITIONS

The kit could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). The components are stable for a maximum of 3 repeated freezing / thawing cycles after the first use of a particular vial.The component must be used before the expiry date or 30 days after the first use of a particular vial (whichever comes first).

TECHNICAL SPECIFICATION

Target Sequence	specific region in M gene and NP gene for Influenza A virus, two specific regions in HA gene for Influenza B virus, specific region in M gene for Respiratory syncytial virus A and B
Analytical Specificity	Influenza A virus, Influenza B virus and Respiratory syncytial virus A and B, 100 %
Analytical Sensitivity (LoD) (with the probability of 95 %)	reaches up to 25.75 cp/ml for Influenza A (on INFA Medium Q Control, Qnostics, using manual extraction GeneProof PathogenFree RNA Isolation Kit), up to 161.64 cp/ml for Influenza A (on INFA Medium Q Control, Qnostics, using automatic extraction croBEE NA16 Nucleic Acid Extraction System), up to 7.25 cp/µl for Influenza B virus (on Amplirun® Influenza B RNA control, Vircell), up to 43.57 cp/µl for Respiratory syncytial virus (on Amplirun® RSV A RNA control, Vircell)
Diagnostic Specificity	100.00% ($CI_{95\%}$: 79.95% - 100.00%)
Diagnostic Sensitivity	92.31% ($CI_{95\%}$: 80.60% - 97.51%)
Validated Specimen	aspirate, BAL, swab, transport medium: Sigma MM*, Sigma Virocult*
Quality Control	regularly tested by QCMD and Instand e.V. External Quality Assessment Panels
Regulatory Status	CE IVD

Quality management system is certified in compliance with the requirements of the standard ČSN EN ISO 13485 ed.2:2016
* only in combination with GeneProof PathogenFree RNA Isolation Kit

INTERFERENCES

The interference testing was performed using negative simulated swabs. The negative samples were spiked with pathogen's positive control at 3x LoD. The tested pathogens were Influenza A Virus, Influenza B Virus and Respiratory Syncytial Virus. Presence of blood, mucin, nasal sprays and drops with different type of active substance (NaCl, oxymetazoline, xylometazoline, phenylephrine) in samples have been tested in the presence and absence of pathogen's nucleic acid. Nasal spray Quixx (Pharmaster) containing 26 g/l NaCl, nasal spray Nasivin Sensitive 0.05% (Merck KGaA & Co) containing 0.5 mg/ml oxymetazoline hydrochloride, nasal spray Olynth HA 0.1% (Famar Health Care Services Madrid, S.A.U.) containing 1 mg/ml xylometazoline hydrochloride and nasal drops Vibrocil (GSK Consumer Healthcare GmbH & Co. KG) containing 2.5 mg/ml Phenylephrine were used. The levels of all markers for testing have been set according to the literature and CLSI guidelines and recommendations of nasal sprays and drops manufacturer.

Tested substance	Tested level(s)	Observed interference	Tested substance	Tested level(s)	Observed interference
Whole blood	2 %	None	Oxymetazoline	0.0625 mg/mL	None
Mucin	60 µg/mL	None	Xylometazolin	0.25 mg/mL	None
Sodium chloride	6.5 mg/mL	None	Phenylephrine	0.375 mg/ml	None

The tested interfering substances were shown not to interfere with GeneProof Flu Multiplex PCR Kit.

METHOD PRINCIPLE

The PCR kit is intended for detection of Influenza A, Influenza B and Respiratory Syncytial Virus (RSV) A/B RNA by the reverse-transcription real-time Polymerase Chain Reaction (PCR) method. The Influenza A detection consists in amplification of specific region in M gene and NP gene. The Influenza B detection consists in amplification of two specific regions in HA gene. The mechanism of duplex targeting ensures maximum sensitivity and specificity of detection. The RSV A/B detection consists in amplification of specific region in M gene. The kit is designed as 4 channel detection assay. The presence of Influenza A is indicated by the increased FAM fluorophore fluorescence. The presence of Influenza B is indicated by the increased Cy5 fluorophore fluorescence. The presence of RSV A/B is indicated by the increased TexRed fluorophore fluorescence. An Internal Control (IC), which is a part of the PCR kit, is used as a control for the whole diagnostic process, i.e. RNA extraction efficiency, reverse-transcription step efficiency (transcription of RNA into cDNA) and PCR amplification efficiency (PCR inhibition). The IC positive amplification is detected in the HEX fluorophore fluorescence channel.

The PCR kit is designed for in vitro diagnostics for qualitative detection and it utilizes the "hot start" technology minimizing non-specific reactions and ensuring maximum sensitivity. It is Ready to Use MasterMix.

SAMPLING AND SAMPLE STORAGE

Swabs: Specimens should be collected using sterile cotton/nylon swabs as a dry swab or placed immediately in viral transport medium. Ship specimens for testing as soon as possible . After collection, prior to and during transport, specimens should be kept at 4 °C for no longer than 72 hours before being processed in the laboratory. For storage periods longer than 72 hours, specimens must be kept at - 70 °C. Aliquot specimens immediately on arrival to the laboratory (aliquot of approximately 0.5 ml, if it is required). Test aliquot immediately ot freeze the at - 70 °C.

BAL, aspirate: Specimens should be collected in sterile vials. If specimens will be examined within 48 hours after collection, keep specimen at 4 °C and ship on wet ice or refrigerant gel-packs, otherwise store frozen at -70 °C and ship on dry ice.

NUCLEIC ACID PURIFICATION

Nucleic acid isolation should be performed by isolation kits available at the market according to protocols for the particular clinical material isolation.

GeneProof PathogenFree RNA Isolation Kit
croBEE NA16 Nucleic Acid Extraction System

When using the PCR kit the IC should be added directly into the sample at the beginning of the extraction process so that in the end 1 µl of the resulting elution volume contains 0.1 µl of the IC:

Elution volume	25 µl	50 µl	100 µl	200 µl
Internal Control	2.5 µl	5 µl	10 µl	20 µl

PCR SETUP

1. Gently vortex and briefly centrifuge the MasterMix and Positive Control tubes.

2. Add 15 µl of MasterMix into PCR tubes.

3. Add 10 µl of the isolated nucleic acid sample or 10 µl of Positive Control into the individual PCR tubes and mix by pipetting. The total reaction mix volume will be 25 µl. WARNING! Reaction volume has to be set to 40 µl. It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. The isolate of negative isolation control with Internal Control should be used in each test. PBS or physiological saline solution, buffer can be used as negative isolation control. The customer has to use his own negative control.

4. Close the tubes, centrifuge shortly, insert them into the device and let them amplify according to the following PCR profile.
Be very careful when handling the Positive Control or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.

AMPLIFICATION PROFILE

Step	Temperature	Time	Data Collection	Cycles
Hold	42 °C	15 min		1
Hold	95 °C	10 min		1
PCR	95 °C	5 s	FAM + HEX + TexRed + Cy5	45
	60 °C	40 s		
	72 °C	20 s		

INSTRUMENTS

GeneProof Flu Multiplex PCR Kit is designed for use with real-time devices from various manufacturers:

croBEE Real-Time PCR System
Applied Biosystems 7500 Real-Time PCR System
CFX96™/ Dx Real-Time PCR Detection System
LineGene 9600 Plus

Required channels: FAM, HEX, Cy5, TexRed
GeneProof diagnostic kits are continually verified with various types of devices. Current list is available at www.geneproof.com or request the list at support@geneproof.com.

SAMPLE ANALYSIS EVALUATION

For evaluation on croBEE Real-Time PCR System and LineGene 9600 Plus devices it is recommended set up S Fitting function. In Cy5 channel (Influenza B virus) positive samples should be evaluated firstly without baseline change. Than it is recommended to set baseline values to 5-20 for Cy5 channel for detection of samples near to limit of detection.

FAM	Cy5	TEX/TexRed/ ROX	HEX/JOE/VIC	Interpretation
+	+	+	+/-	Influenza A virus, Influenza B virus and RSV positive
+	-	+	+/-	Influenza A virus and RSV positive
+	+	-	+/-	Influenza A virus and Influenza B virus positive
+	-	-	+/-	Influenza A virus positive
-	+	+	+/-	Influenza B virus and RSV positive
-	-	+	+/-	RSV positive
-	+	-	+/-	Influenza B virus positive
-	-	-	+	Influenza A virus, Influenza B virus and RSV negative
-	-	-	-	Invalid

RSV = Respiratory Syncytial Virus

WARNING

A single valid Instruction for Use for a specific kit is included in the package or to be requested for the particular lot from the manufacturer. Use only combination of components from the specific PCR kit. The kit should be disposed of after use according to the current legal regulations considering the fact that the kit does not contain any dangerous, infectious or toxic components that would be subject to special safety regulations, and the packaging materials are made of paper and polypropylene. If you have any questions please contact our Customer Service.

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