EURx® MOLECULAR BIOLOGY PRODUCTS

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Product intended for laboratory/veterinary use, in vitro diagnostic use.

Influenzavirus A qRT-PCR Detection Kit

(diagnostic kit detecting influenzavirus A genome fragments)

Influenzavirus A qRT-PCR Detection Kit is designed to detect influenzavirus A specific RNA sequences in both human and animal (including avian) samples. The purified viral genetic material is amplified by real-time RT-PCR and detected with influenzavirus A specific probes labeled with the FAM fluorescent dye. Virus identification is based on two highly conserved regions in segment 7 of the virus encoding M1, M2 proteins, and one region in segment 3 encoding the PA protein that is part of the RNA polymerase complex. In addition, the kit includes an internal control (primers, probe labeled with HEX dye, exogenous fragment of nucleic acid - Internal Control), which allows for simultaneous verification of RNA isolation and PCR inhibition procedures.

Kit contents

Składnik	**Cat. no. E0459—01** 100 reactions, 25 µl each	**Cat. no. E0459—02** 500 reactions, 25 µl each
IAV Buffer Mix (2x) * brown tube	2 x 625 µl	10 x 625 µl
IAV Enzyme Mix orange cap	100 µl	5 x 100 µl
Internal Control blue cap	2 x 500 µl	10 x 500 µl
Positive Control** black cap	200 µl	1000 µl
RNase free Water transparent cap	1200 µl	5 x 1200 µl

Storage

All components of the kit should be stored at -20° C.
* Avoid multiple thawing and freezing (> 2) as this may reduce assay sensitivity. The reagent should be frozen in smaller aliquots if it is used only occasionally.
**Positive Control should be stored separately, away from other kit components.

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ROX reference dye

ROX reference dye contained in the IAV Buffer Mix (2x) of the Influenzavirus A qRT-PCR Detection Kit enables the normalization of fluorescence in some real-time PCR devices. The use of ROX reference dye is mandatory for all Applied Biosystems thermal cyclers and optional for Agilent thermal cyclers. ROX compensates for changes in fluorescence signal between wells due to slight variations in reaction volume and fluctuation in fluorescence. The presence of ROX dye does not interfere with real-time PCR on devices that do not require this reference dye.

Preparation of RNA sample

RNA from swabs and tissue samples should be isolated using kits dedicated to viral RNA purification. Follow the instructions recommended by the kit manufacturer.

Internal Control

The kit includes an internal control system that allows to monitor the RNA isolation process, as well as the inhibition of the PCR process itself.

The internal control system consists of:

- primers and probe labeled with HEX dye present in the IAV Buffer Mix (2x),
- exogenous nucleic acid fragment contained in Internal Control.

Internal Control can be added at the RNA isolation stage (point A) - then it is both a control of the RNA isolation and PCR inhibition, or only at the PCR stage (point B) - to assess the quality of the purified RNA and possible PCR inhibition.

A. Add Internal Control to the lysis buffer or to the mixture of lysis buffer and sample material (do not add to the sample material directly) at a ratio 0.1 µl per 1 µl elution volume. For example, 5 µl of Internal Control should be added initially using 50 µl elution volume. It is recommended to use carrier RNA when isolating RNA from cell-free body fluids and material low in RNA content. Carrier RNA increases the efficiency of RNA binding to silica membranes in the presence of small amounts of genetic material in the sample.

B. Add 0.5 µL of the Internal Control for every 25 µL of PCR reaction.

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Protocol
Preparation of PCR reaction:

Component	Negative Amplification Control-NAC	Negative Extraction Control-NEC	Positive Extraction Control-PEC	RNA sample	Positive Control IAV-TPC
IAV Buffer Mix (2x)	12.5 µl	12.5 µl	12.5 µl	12.5 µl	12.5 µl
IAV Enzyme Mix	1 µl	1 µl	1 µl	1 µl	1 µl
Internal Control (optional)	-	-	-	0.5 µl	0.5 µl
Positive Control	-	-	-	-	5 µl
Sample of extracted H_2O/	-	5 µl	-	-	-
extracted Internal Control/	-	-	5 µl	-	-
extracted RNA	-	-	-	5 µl	-
RNase free Water	to 25 µl	to 25 µl	to 25 µl	to 25 µl	to 25 µl
Total volume	25 µl	25 µl	25 µl	25 µl	25 µl

Notes:

1. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
2. Thaw, gently vortex and briefly centrifuge all solutions. Keep IAV Enzyme Mix on ice, while the reactions can be prepared at room temperature.
3. Prepare PCR reactions according to the table above.
4. Add:

- water (negative amplification control-NAC),
- 5 µl of water purified according to the RNA isolation procedure (negative extraction control-NEC),
- 5 µl of Internal Control purified according to the RNA isolation procedure (positive extraction control-PEC),
- 5 µl of purified RNA sample,
- 5 µl of Positive Control, IAV-TPC.

5. IAV-TPC gives a signal in the FAM channel only, when no Internal Control was added to the reaction. To obtain a signal in both the FAM and HEX channels, add 0.5 µl of Internal Control to the reaction. Increasing the PCR reaction volume for the IAV-TPC by 0.5 µL to 25.5 µL (when preparing one reaction mix for RNA samples and controls) does not adversely affect the efficiency of the PCR reaction.
6. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
7. Place the samples in the thermal cycler programmed according to the table below.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of cycles
Reverse transcription	50°C	15 min	1
Initial denaturation	95°C	2 min	1
Denaturation	95°C	10 s	40-45
Annealing/extension	60°C	45 s	

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Notes:

1. Run PCR reactions using standard ramp rate (standard mode).
2. Fluorescence detection should be set on the end of the extension step and executed using FAM and HEX channels. FAM channel detects influenzavirus A RNA and HEX channel detects the inner control.

Results Interpretation:

Sample type	FAM channel	HEX channel	Result
Negative Amplification Control-NAC	-	-	correct
Negative Extraction Control-NEC	-	-	correct
Positive Extraction Control-PEC	-	+	correct
Positive Control, IAV-TPC (- Internal Control) or (+ Internal Control)	+ +	- +	correct
1	-	+	negative IAV^-
2	+	+	positive IAV^+
3	+	-	positive IAV^+
4	-	-	incorrect

Notes:

1. A test can be considered valid/correct if:
   - negative amplification control-NAC does not give a signal in both the FAM and HEX channels,
   - negative extraction control-NEC does not give a signal in both the FAM and HEX channels,
   - positive extraction control (when Internal Control was purified according to the RNA isolation procedure) gives a signal only in the HEX channel ($C_T \leq 34$),
   - positive control, IAV-TPC gives a signal only in the FAM channel when no Internal Control was added to the reaction ($C_T \leq 33$) or in both the FAM and HEX channels when Internal Control was added to the reaction (C_T in both channels ≤ 33).
2. The sample is positive (IAV^+) and the test is valid if:
   - the conditions of point 1 are met,
   - the sample gives a signal in the FAM channel ($C_T \leq 40$) and in the HEX channel for the Internal Control ($C_T \leq 34$).

   In exceptional circumstances, when the reaction is strongly positive (IAV^+), the sample may give a weak signal or no signal in the HEX channel. The reason for this is competition between IAV and Internal Control amplification reactions.
3. The sample is negative (IAV^-) and the test is valid if:
   - the conditions of point 1 are met,
   - the sample gives a signal only in the HEX channel ($C_T \leq 34$).
4. The presence of a fluorescence signal in the HEX channel for a given sample indicates that the RNA isolation process and PCR reaction were correct (in the case of adding Internal Control to the RNA isolation process), or only that the PCR reaction was not inhibited (in the case of adding Internal Control to the PCR reaction mixture). Very high C_T values (> 34) in the HEX channel may indicate a partial inhibition of the PCR reaction. In turn, the complete absence of a signal in the HEX channel with the simultaneous lack of a signal in the FAM channel may indicate complete inhibition of the PCR reaction or failure to add RNA to the PCR reaction with the simultaneous addition of Internal Control in the RNA isolation process. In this case, it is recommended to dilute the sample 10 times or repeat the RNA isolation and rerun the PCR reaction.
5. Lack of signal for the IAV-TPC indicates an error in PCR preparation.

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