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For laboratory/veterinary use only, for *in vitro* diagnostic

Avian IAV qRT-PCR Kit

(diagnostic kit detecting fragments of the influenza A virus genome in birds)

The Avian IAV qRT-PCR Kit is intended to detect influenza A virus-specific RNA sequences in avian samples. The purified viral genetic material is amplified using real-time RT-PCR and detected using probes specific to the influenza A virus, labeled with the fluorescent FAM dye. The virus is identified based on a highly conserved region in segment 7 of the virus encoding the M1, M2 proteins and the region in segment 3 encoding the PA protein, which is part of the RNA polymerase complex. Additionally, the kit contains primers and a probe labeled with HEX dye enabling the detection of a fragment of the avian ACTB gene, encoding beta-actin and constituting an endogenous internal control of the correctness of RNA isolation and PCR reaction.

Kit contents

Component	**Cat. no. E0445–01** 100 reactions of 25 µl	**Cat. no. E0445–02** 500 reactions of 25 µl
IAV Master Mix brown tube	4 x 500 µl	20 x 500 µl
Positive Control black cap	200 µl	1000 µl
RNase free Water clear cap	1200 µl	5 x 1200 µl

Storage:

The kit should be stored in the dark at –20°C. Avoid repeated thawing and freezeing (>2x), due to possible reduced sensitivity.

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Types of RNA samples, RNA sample preparation

RNA from swabs (oropharyngeal, tracheal, cloacal), tissue samples, organs (organ homogenates, intestines) should be isolated using kits dedicated to the purification of viral RNA. Please follow the instructions recommended by the kit manufacturer. Due to the high sensitivity of the kit, both single and pooled samples of up to 10 individual can be tested.

The reference dye ROX

The passive ROX reference dye included in the master mix enables fluorescence normalization in particular cyclers. The use of ROX dye is required for all Applied Biosystems real-time PCR cyclers and optional for Agilent cyclers. ROX compensates for differences in fluorescence signal between wells caused by small differences in reaction volume and fluorescence fluctuations. ROX is not involved in the PCR reaction and does not interfere with real-time PCR on any instrument.

IAV Standard Template

The IAV Standard Template (cat. no. E0458) is a complementary product to the Avian IAV qRT-PCR Kit and can be ordered additionally. IAV Standard Template is a reference solution containing a synthetic fragment of influenza A virus nucleic acid. The synthetic fragment includes: the region of segment 7 of the virus encoding the M1, M2 proteins and the region of segment 3 encoding the PA protein, which is part of the RNA polymerase complex. The IAV Standard Template can be used as a positive control for influenza A virus extraction in the Avian IAV qRT-PCR Kit.

Procedure
Preparation of PCR reaction:

Component	Negative amplification control -NAC	Negative extraction control-NEC	RNA sample	Positive Control IAV -TPC
IAV Master Mix	20 µl	20 µl	20 µl	20 µl
RNase free Water	5 µl	-	-	-
Purified water sample or negative sample IAV$^-$	-	5 µl	-	-
RNA Sample	-	-	5 µl	-
Positive Control	-	-	-	5 µl
Volume	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 the kit components on ice. Avoid multiple thawing and freezing (> 2x) of IAV Master Mix, as this may reduce the sensitivity. Set up PCR reactions at room temperature. Use IAV Master Mix allows room temperature reaction setup.
3. Dispense 20 µl of IAV Master Mix into PCR tubes or plates.
4. Add:

- 5 µl of water (negative amplification control-NAC),
- 5 µl of water or negative sample IAV$^-$ purified according to RNA extraction protocol (negative extraction control-NEC),
- 5 µl of purified RNA (RNA sample),
- 5 µl of Positive Control IAV-TPC.

6. Centrifuge briefly to settle down the reaction components and remove bubbles. Place the samples in the cycler and start the program prepared according to the table below. Perform data analysis.

Real-time PCR Protocol:

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

Notes:

1. Run PCR reactions using standard ramp rate (standard mode) only.
2. Fluorescence analysis in the FAM and HEX channels should be performed by the device at the end of the extension step. IAV RNA is detected in the FAM channel and avian beta-actin gene DNA is detected in the HEX channel.

Data Analysis and Interpretation:

Sample type	FAM channel	HEX channel	Sample result
Negative amplification control-NAC	-	-	correct
Negative extraction control-NEC	-	-/+	correct
Positive Control IAV-TPC	+	+	correct
Sample 1	-	+	negative IAV^-
Sample 2	+	+	positive IAV^+
Sample 3	+	-	positive IAV^+
Sample 4	-	-	incorrect

Notes:

1. For the assay to be valid:

- negative amplification control-NAC does not yield a signal in both FAM and HEX channels,
- negative extraction control-NEC does not yield a signal in both FAM and HEX channels (for purified water) or yield a signal only in HEX channel (for negative sample IAV^-),
- Positive Control IAV-TPC yields a signal in both FAM and HEX channels.

1. RNA sample is positive (IAV^+) and the assay is valid if:

- the cryteria in the first point are met,
- sample yields a signal at least in FAM channel.

  Exceptionally very high concentrations of IAV RNA in the sample may lead to a reduced HEX signal or no HEX signal at all due to the competition with the endogeneous internal control.

3. RNA sample is negative (IAV^-) and the assay is valid if:

- the cryteria in the first point are met,
- sample yields a signal only in HEX channel.

4. A positive HEX signal for the sample means that the RNA extraction and amplification were successful. High C_T values (>35) for internal control may indicate partial PCR reaction inhibition. In turn, lack of a signal at all in the HEX and FAM channel simultaneously may indicate complete PCR reaction inhibition or that no RNA was added to the test sample. In this case it is recommended to dilute 10 times the RNA sample or repeat the RNA extraction and run the PCR reaction again.
5. Absence of a signal for the Positive Control IAV-TPC indicates to an error, which could be due to incorrect setup of the PCR reaction.