

Instruction Manual

INFLUCHECK AVIAN VIRUS
Real time PCR kit

Code EVR021050 Format 50 reactions

Real Time PCR kit for Screening & Typing of Avian A Influenza
Virus (H5/H7)

For in Vitro Use Only

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INTENDED USE

Influchek Avian Virus real time PCR kit (cod EVP021050) is an *in vitro* veterinary nucleic acid amplification test for screening of Avian influenza A virus RNA in clinical specimens (tissues, eggs, mucosal swabs and faeces) and for their genotyping (H5 or H7).

PRINCIPLE OF ASSAY

The kit is based on PCR in Realtime technique with a TaqMan FAM/HEX marked probes. It is complete of all amplification reagents for screening and genotyping.

KIT FORMAT

The **Influchek Avian Virus real time PCR kit** is suitable for 50 reactions.

KIT CONTENTS

Part N° 1 – “**Screen**”: amplification module for the screening of type A; (Cod EVR019050)

- Amplification mix, 0,5ml
- Oligo mix, 0,5ml (specific for the screening)
- Positive control, 50 µl
- Negative control, 50 µl

Part N° 2 – “**Type** ”: amplification module for the typing (H5 and H7); (Cod EVR020050)

H5/H7

- Amplification mix, 1 ml
- Oligo mix, 0,5ml (specific for the typing H5)
- Oligo mix, 0,5ml (specific for the typing H7)
- Positive control, 50 µl (H5 positive)
- Positive control, 50 µl (H7 positive)

WARNING: Positive and negative controls are ready for the amplification (they must not be used in the retro-transcription reaction)

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA extraction kit
- Retrotranscription kit
- Real Time Thermalcycler (the kit is suitable for the most common commercially available equipment)
- PCR workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl)
- Tube racks
- Vortex

Some suggested protocols for RNA extraction from clinical specimens (tissues, eggs, mucosal swabs and faeces) are listed below:

Eggs:

- Pipet the sample in a sterile tube using a sterile filter tip and mix with the Lysis Buffer included in the RNA extraction kit used.

Tissues:

- Take 2-3 mm³ of the tissue with a knife and put it in a mortar;
- Add a small quantity of quartz powder to grind it finely;
- Add 300 µl of PBS, homogenize and transfer it into a 1,5ml tube;

- Put all equipment used in virkon 1% or sodium ipochlorite;
- Centrifuge the sample for 30 sec at the highest speed;
- Pipet the supernatant in a sterile tube, with a sterile filter tip, and mix it with the Lysis Buffer included in the RNA extraction kit used.

Mucosal swabs:

- Put 1 ml of sterile PBS in a 15ml sterile tube;
- Blend the swab in PBS very carefully;
- Homogenize this suspension with a vortex;
- Pipet the supernatant in a sterile tube, with a sterile filter tip, and mix it with the Lysis Buffer included in the RNA extraction kit used.

Faeces:

- Take 1g of faeces and add 1ml of PBS (ratio 1:1 = gr faeces : ml PBS);
- Homogenize this suspension with a vortex and transfer 1ml of the homogenate into a 1,5ml tube;
- Centrifuge the sample for 30 sec at the highest speed;
- Pipet the supernatant in a sterile tube, with a sterile filter tip, and mix it with the Lysis Buffer included in the RNA extraction kit used.

SCREENING AMPLIFICATION PROTOCOL (“Screen” MODULE)

1. Prepare required quantity of PCR tubes for samples and controls (negative and positive)
2. Depending on how many samples had to be tested, make a MASTER mix with the Amplification mix and the Oligo mix and performed the reaction following the listed below indications:

NB: for example for 5 samples (plus the two controls) make a MASTER mix of 160 µl (calculated a volume excess!)

for a single reaction

Amplification Mix	10 µl
Oligo mix	10 µl
Sample cDNA/C+/C-	5 µl

3. Add 20 of the REAGENT mix to each tube
4. Add 5 µl of the sample cDNA to the marked tube
5. Add 5 µl of the positive control to the marked tube
6. Add 5 µl of the negative control to the marked tube
7. Set the thermal cycler equipment on the FAM fluorescence channel
8. Set the following amplification protocol

TIME	TEMPERATURE	CYCLES
10 min	95°C	1
15 sec	95°C	45
1 min	60°C	

9. Insert tubes in the thermocycler and start the run

INTERPRETATION OF RESULTS

Fluorophore (FAM) sample	Fluorophore (FAM) control	RESULTS
$C_t > 0$	$C_t > 0$	POSITIVE
$C_t = 0$	$C_t > 0$	NEGATIVE
$C_t = 0$	$C_t = 0$	/

TYPING AMPLIFICATION PROTOCOL (“Type” MODULE)

H5- Amplification

1. Prepare required quantity of PCR tubes for samples and the controls (negative and positive). NB: negative control is not supplied (use milliQ water)
2. Depending on how many samples had to be tested, make a MASTER mix with the Amplification mix and the **Oligo mix (H5)** and performed the reaction following the listed below indications:

NB: for example for 5 samples (plus the two controls) make a MASTER mix of 160 μ l (calculated a volume excess!)

for a single reaction

Amplification Mix	10 μ l
Oligo mix	10 μ l
Sample cDNA/C+/C-	5 μ l

3. Add 20 of the REAGENT mix to each tube
4. Add 5 μ l of the sample cDNA to the marked tube
5. Add 5 μ l of the positive control to the marked tube
6. Add 5 μ l of the negative control to the marked tube
7. Set the thermal cycler equipment on the FAM fluorescence channel
8. Set the following amplification protocol

TIME	TEMPERATURE	CYCLES
10 min	95°C	1
15 sec	95°C	45
1 min	54°C	

9. Insert tubes in the thermocycler and start the run

INTERPRETATION OF RESULTS

Fluorophore (FAM) sample	Fluorophore (FAM) control	RESULTS
$C_t > 0$	$C_t > 0$	POSITIVE
$C_t = 0$	$C_t > 0$	NEGATIVE
$C_t = 0$	$C_t = 0$	/

TYPING AMPLIFICATION PROTOCOL (“Type” MODULE)

H7- Amplification

1. Prepare required quantity of PCR tubes for samples and the controls (negative and positive). NB: negative control is not supplied (use milliQ water)
2. Depending on how many samples had to be tested, make a MASTER mix with the Amplification mix and the ***Oligo mix (H7)*** and performed the reaction following the listed below indications:

NB: for example for 5 samples (plus the two controls) make a MASTER mix of 160 μ l (calculated a volume excess!)

for a single reaction

Amplification Mix	10 μ l
Oligo mix	10 μ l
Sample cDNA/C+/C-	5 μ l

3. Add 20 of the REAGENT mix to each tube
4. Add 5 μ l of the sample cDNA to the marked tube
5. Add 5 μ l of the positive control to the marked tube
6. Add 5 μ l of the negative control to the marked tube
7. Set the thermal cycler equipment on the HEX fluorescence channel
8. Set the following amplification protocol

TIME	TEMPERATURE	CYCLES
10 min	95°C	1
15 sec	95°C	45
1 min	54°C	

9. Insert tubes in the thermocycler and start the run

INTERPRETATION OF RESULTS

Fluorophore (HEX) sample	Fluorophore (HEX) control	RESULTS
$C_t > 0$	$C_t > 0$	POSITIVE
$C_t = 0$	$C_t > 0$	NEGATIVE
$C_t = 0$	$C_t = 0$	/

RELATED EUROCLONE PRODUCTS

Influchek Avian Virus PCR end point- Cod. EVP018055
OMNIZOL – RNA EXTRACTION- Cod EMR060100