Labor Diagnostik
Leipzig

VIROTYPE® Influenza A

Instructions for Use

Real-time RT-PCR Test Kit for Detection of Influenza A Virus

In vitro Diagnostic Kit for Birds and Swine

Registered in Accordance with §17c of the German Law on Animal Diseases
Registration No.: FLI-B 538

VIROTYPE® Influenza A	25	reactions	Cat. No.	05-301/25
VIROTYPE® Influenza A	96	reactions	Cat. No.	05-301/96
VIROTYPE® Influenza A	480	reactions	Cat. No.	05-301/480

Applications

The product group VIROTYPE® comprises test systems for the identification of viral pathogens by real-time PCR.
VIROTYPE® Influenza A is a real-time RT-PCR test kit for the detection of Influenza A virus. Sample materials are oropharyngeal, tracheal and cloacal swabs (individual or pooled), fecal samples or tissue samples from birds, nasal swabs (individual or pooled), bronchoalveolar lavage fluid (BALF) and tissue samples from swine and supernatant of cell culture.

Production & Support:

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General Information

Viruses of the genus *Influenzavirus A* belong to the family *Orthomyxoviridae*. They occur in high genetic diversity and a wide range of virulence.

Influenza A viruses are grouped into low and highly pathogenic strains. Waterfowl are the natural reservoir of low-pathogenic avian influenza viruses (LPAIV). Highly pathogenic avian influenza viruses (HPAIV) belong to subtypes H5 or H7 and may cause fowl plaque in domestic poultry with high economic losses. The subtypes H1N1, H1N2 and H3N2 of Influenza A virus can also cause infections of the respiratory tract in swine.

The high sensitivity of VIROTYPE® Influenza A allows the early detection of the pathogen in samples of birds and swine. In addition the novel A/H1N1 (2009) Influenza A virus is detected.

Description of the Test Principle

VIROTYPE® Influenza A includes all reagents for the detection of Influenza A virus RNA as well as a positive and a negative control.

Reverse transcription (RT) of the viral RNA and subsequent amplification of the cDNA by Polymerase Chain Reaction (PCR) are performed in one tube; this reduces the risk of contaminations to a minimum. The reporters of the probes will emit fluorescence in proportion to the amount of amplificate produced. Thus, the reaction can be monitored in real-time (real-time PCR).

VIROTYPE® Influenza A uses two specific combinations of primer with probe, one for Influenza A virus RNA giving FAM fluorescence and one for the control RNA (internal control) giving HEX fluorescence. As assay internal control mRNA of ß-actin housekeeping gene is amplified. This guarantees the control of extraction as well as amplification.

Content

		25 reactions	96 reactions	480 reactions
1	**Influenza A-Mix** (orange cap) **includes primers, probes and enzymes**	520 µl	2 x 980 µl	6 x 1.625 ml
2	**Positive Control** (red cap)	25 µl	70 µl	2 x 50 µl
3	**Negative Control** (blue cap)	25 µl	70 µl	2 x 50 µl

Storage and Shelf Life

Immediately upon receiving store the test kit at -20 °C, protected from light. Under these conditions the test kit is stable until the indicated expiration date. Do not use the reagents past expiration date.

Influenza A-Mix and Positive Control should not be thawed and frozen more than 3 times. In case of frequent use of the test kit aliquoting is recommended.

Additional Material and Equipment Required

- RNA extraction kit
- Pipettes (0.5 µl – 1000 µl)
- Sterile filter tips (aerosol resistant)
- Sterile 1.5 ml reaction tubes
- Bench-top centrifuge
- PCR tubes: 96-well reaction plate or reaction tubes
- Optical sealing film or covers
- Real-time PCR thermal cycler: e.g. Mx3000P, Mx3005P (Stratagene/ Agilent), ABI 7500 (Applied Biosystems), CFX96 System (BioRad), Rotor-Gene (QIAGEN) or similar

Trademarks and Patents

VIROTYPE® is a registered trademark. Patent rights protect PCR.
THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER CERTAIN ROCHE PATENTS TO USE IT FOR PROVIDING VETERINARY IN VITRO DIAGNOSTIC TESTING. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

Precautions and Warnings

The test should only be performed by persons qualified for laboratory work. The components of the test kit may not be contaminated or mixed with components from other batches. Sample preparation and amplification should be carried out in separate rooms. Take appropriate safety measures for working in laboratories and stick to GLP rules. Wear gloves all the time.

All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Please stick rigorously to this protocol (page 5)!
For veterinary use only.

Sample Material

VIROTYPE® Influenza A can be used for the detection of Influenza A virus RNA from oropharyngeal, tracheal and cloacal swabs, fecal samples or tissue samples from birds, nasal swabs, bronchoalveolar lavage fluid (BALF) and tissue samples from swine and supernatant of cell culture. Due to the high sensitivity of the test, pools of up to 10 individual swab samples can be analysed.

RNA Extraction

For the isolation of Influenza A virus RNA following extraction kits can be used:

QIAamp® Viral RNA Mini (QIAGEN)	swab samples, fecal samples
NucleoSpin® RNA Virus (Macherey & Nagel)	swab samples, fecal samples
Invisorb Spin Virus RNA Mini Kit (Invitek)	swab samples, fecal samples
MagMAX Viral RNA Isolation Kit (Applied Biosystems)	swab samples
RNeasy® Kit (QIAGEN)	tissue samples
RNeasy® Fibrous Tissue Kit (QIAGEN)	tissue samples
NucleoSpin® RNA II (Machery & Nagel)	tissue samples
or comparable, validated systems	

Store the isolated RNA at -70 °C (-20 °C or below) in case the real-time RT-PCR is not performed immediately after RNA extraction.

Influenza A real-time RT-PCR Protocol

Please read the entire protocol before starting the test procedure. RNA is unstable, please perform this protocol uninterrupted.

Before use, thaw the reagents at room temperature (18-25 °C) protected from light, mix and spin shortly. Perform all pipetting steps on ice.

Calculate the number of reactions to be performed (samples + controls) plus a reserve. Perform at least one Positive and one Negative Control for each run.

1. Mix the Influenza A-Mix (orange cap) and spin shortly, take the volume needed and pipette it into a sterile reaction tube.

   Immediately return not required Influenza A-Mix at -20 °C in the dark.

2. Add 20 µl of Influenza A-Mix into each PCR tube.

3. Add 5 µl of the isolated RNA or of controls (red and blue cap) and mix. Thus, the final volume of a test is 25 µl.

4. Immediately cover the PCR tubes with optical sealing film, or close caps, respectively. Spin shortly if necessary.

Use the following filter settings for reporter and quencher in the software of your thermal cycler:

	Reporter	Quencher
Influenza A	**FAM**	TAMRA
Internal control	**HEX/**JOE[1]	TAMRA
Passive reference[2]	ROX	

1 use option available in your thermal cycler
2 internal reference using the ABI PRISM® Sequence Detection Systems

Influenza A Real-time RT-PCR-Program:

45 °C	10 min	
95 °C	10 min	
95 °C	15 sec	40 cycles
60 °C	60 sec	Measure at the end of this step

(Total time with Stratagene Mx3000P: 1 h 41 min)

Alternatively the standard **VIROTYPE®-Program** can be used if the assay is performed in parallel with other VIROTYPE® products (VIROTYPE® BTV Plus, VIROTYPE® BVDV and VIROTYPE® CSFV) in the same thermocycler:

VIROTYPE® Real-time RT-PCR-Programm:

50 °C	20 min	
95 °C	15 min	
95 °C	30 sec	40 cycles
57 °C	45 sec	Measure at the end of this step
68 °C	45 sec	

(Total time with Stratagene Mx3000P: 2 h 29 min)

Test Validation

For the assay to be valid the FAM and HEX fluorescence signals of the Positive Control should give a Ct-value less than 35 (Ct < 35).
The Negative Control does not have a fluorescence signal.

Interpretation of the Results

1 A FAM fluorescence signal is measured:

→ Positive result, the sample contains Influenza A virus RNA.

In this case, a HEX fluorescence signal may be dispensable, because very high concentrations of Virus-RNA may compete with the internal control giving a reduced HEX signal or no HEX signal at all.

2 Only a HEX fluorescence signal (internal control) is measured:

→ Negative result, the sample does not contain Influenza A virus RNA.

The internal control is measured through HEX fluorescence, complete PCR inhibition and incorrect extraction is thereby excluded.

3 No fluorescence signal is detected:

→ The test is not valid, no diagnosis possible.

The PCR was inhibited or the sample extraction was incorrect. It is recommended to retest the respective individual samples in nuclease free water (e.g. diluted 1:5), to repeat the RNA extraction or repeat the whole test procedure starting with new sample material.

Analysis of supernatant of cell culture:

→ Negative result, the sample does not contain Influenza A virus RNA.

However, no information about PCR inhibition or incorrect extraction is given.

Notes .....