

## INgene q Influenza A virus

R.10.IFA.K.5/100

qPCR kit for the detection of Influenza A virus in biological samples. *Real Time PCR (TaqMan technology).*

### KIT FEATURES

#### APPLICATION

INgene q Influenza A virus is a real-time PCR kit suited for the detection of Influenza A virus (**Influenza A**) RNA in biological samples from **Swine, Equine, Poultry** of clinical interest. The assay has been optimized and specifically tested for **lungs, respiratory secretions, oral fluids** and strains/vaccines.

*Intended use for research.*

#### TECHNICAL BASE

1. INgene q Influenza A virus is an assay for amplification and detection of the Influenza A genetic material based on the polymerase chain reaction (PCR) and hydrolysis fluorescent probes (TaqMan type). The assay consists of a duplex real-time PCR in a one-well format.
2. This assay contains specific primers and a probe labelled with FAM fluorochrome for the detection of **Influenza A**. In addition, the mix includes an EC that is amplified with specific primers and detected with a probe labelled with HEX/VIC, allowing identification of false negatives due to poor extraction and/or PCR inhibition.
3. Positive Control provided with the kit allows the optional relative/absolute quantification of the genetic material of interest, by performing a standard curve.
4. The kit has been validated in the following PCR platforms: QuantStudio 5 Dx System from Applied Biosystems, and CFX96™ Real-Time PCR System from Bio-Rad. It is compatible with other thermocyclers, if they have the appropriate fluorescence channels, but Cq values may vary among them. Unselect ROX as passive reference in those equipment that have this channel selected by default.

#### RESULTS INTERPRETATION

At the end of the run, results should be analysed with the thermocycler software following manufacturer instructions. Before interpreting the samples result, check that the result of the Positive, Negative and Extraction controls are as expected.

SAMPLE (FAM) Positive if Cq ≤ 38 <sup>1</sup>	POSITIVE CONTROL (FAM)	NEGATIVE CONTROL (FAM)	SAMPLE Endogenous control (HEX)	ASSAY RESULT
POSITIVE	POSITIVE	NEGATIVE	DO NOT CONSIDER	VALID
NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	VALID
NEGATIVE	POSITIVE	NEGATIVE	<b>NEGATIVE</b>	NOT VALID
POSITIVE/NEGATIVE	<b>NEGATIVE</b>	NEGATIVE	POSITIVE/NEGATIVE	NOT VALID
POSITIVE/NEGATIVE	POSITIVE	<b>POSITIVE</b>	POSITIVE/NEGATIVE	NOT VALID

<sup>1</sup> In case of **strains/microbiological isolates**, the sample is considered positive if Cq ≤ 30.

### ASSAY VALIDATION

#### VALIDATION DATA

- a) Three different Influenza A commercial vaccines: Gripork® (H1N1 strain OLL, H3N2 strain A) HIPRA, RespiPork Flu3® (H1N1 Haselünne strain, H1N2 Bakum strain, H3N2 Bakum-IDT strain) IDT Biologika, Fluvac T® (H7N7 strain Praga, H3N8 strain Lexington, H3N8 strain Kentucky) PFIZER, resulted positive.
  - b) A total of 31 related microorganisms including bacteria (n=26) such as Mycoplasmataceae, Pasteurellaceae, Streptococcaceae, etc., virus (n=4) and fungi (n=1) was evaluated as an specificity panel. All of them resulted negative.
  - c) A panel of 174 clinical cases from pigs with potential diagnosis of respiratory disease was evaluated with this kit. Samples included lungs (n=107), bronchial alveolar lavage fluid (n=28), tracheobronchial brush samples (n=11), oral fluids (n=28). Influenza A virus was detected in 11% (19/174) of these samples, mainly in oral fluids (14%), bronchial alveolar lavages(11%) and lungs (10%).
  - d) The assay INgene q Influenza A virus was tested in the International Proficiency Testing Scheme (PTS) for avian influenza virus (AIV) RNA detection on FTA card (2022) organized by Royal GD, Deventer the Netherlands. The PTS samples included the following prototype strains: LP H7N1: A/parrot/N.Ireland/vf-73-67/73, LP H6N2: A/turkey/Massachusetts/65, LP H5N1: A/Chicken/Netherlands/SP00153/2014, LP H5N2: A/Chicken/Belgium150/99 soncke99/150 v6, LP H9N2: A/Chicken/Saudi Arabia/SP02525/3AAV2000; LP H5N3, A/Mallard/Sweden/Eskilstuna/05, HP H5N8: A/Chicken/Netherlands/SP00213/2017. Sample analysis performed with the kit INgene q Influenza A virus allowed the correct assignment of all the PTS samples.
- We studied the reportable range for this qPCR assay using a purified specific synthetic oligonucleotide (positive control).
  - This qPCR kit can identify Influenza A from clinical samples and vaccine/field strains.
  - Maximum quantification limit of Influenza A ≥ 109 copies/reaction.
  - Minimum quantification limit of Influenza A = at least 50 copies/reaction.

#### KIT COMPOSITION

- Assay mix tube
- Positive Amplification Control
- Nuclease free water

**EXPIRATION: 18 MONTHS**  
Stored ≤ -18°C



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