

ViroReal® Typing Kit InfA H5N8

Order No.: DVET003

Unit: 50 reactions

For veterinary use only



Kit contents:

- Separate detection assays for Influenza A H5 and N8
- SYBR® Green RT-PCR Mix for one-step reverse transcription real-time PCR with subsequent melt curve analysis containing a highly purified Taq Polymerase for hot-start PCR, dNTPs with dUTP, SYBR® Green dye, ROX™ dye (passive reference), optimized buffer components and a Reverse Transcriptase with RNase inhibitor
- Separate RNA positive controls for Influenza A H5 and N8
- Nuclease-free water

Background:

Influenza A virus is a single-stranded, negative-sense RNA virus of the family Orthomyxoviridae. Of the three Influenza genera (A, B, C), only Influenza A is known to infect birds. Avian Influenza is further characterized based on the presence of haemagglutinin/neuraminidase subtypes. Various strains, especially of H5N1 and H5N8 genotype, are responsible for outbreaks in domestic ducks and migratory birds in East Asia, Northern America, parts of Europe and the Middle East. Further spreading around the globe is possible through migratory birds. Virulence potential of a certain strain can vary from mild to severe due to high sequence variability.

Description:

ViroReal® Typing Kit InfA H5N8 is a one-step reverse transcription real-time PCR assay followed by a melt curve step, containing two PCR primer sets for the detection of RNA of avian influenza A H5N8.

ViroReal® Typing Kit InfA H5N8 detects the haemagglutinin (H) and neuraminidase (N) genes of avian influenza A virus, subtype H5N8. Amplification curves and specific melt curves for the targets H5 and N8 indicate the amplification of genotype specific RNA. It should be emphasized, however, that the viral genome consists of segmented RNA fragments highly prone to sequence variation. The test is based on currently published viral motives of known virulence, but constant re-arrangement makes it impossible to cover all potential relevant strains.

PCR-platforms:

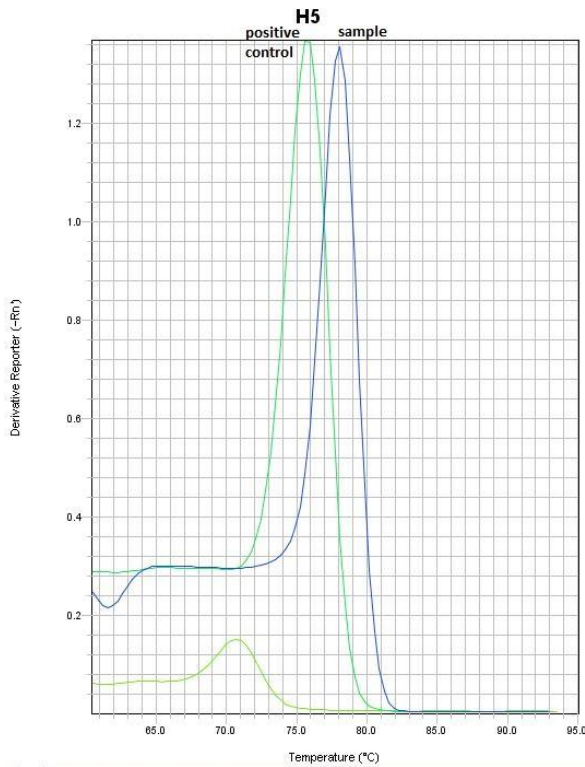
This test is based on an intercalating dye using one-step reverse-transcription real-time PCR and was developed and validated for the Applied Biosystems® 7500 (Fast) instrument (Thermo Fisher Scientific), but is also suitable for other real-time PCR instruments. This test allows the rapid typing of avian influenza H5N8 from RNA samples.

Sensitivity and specificity:

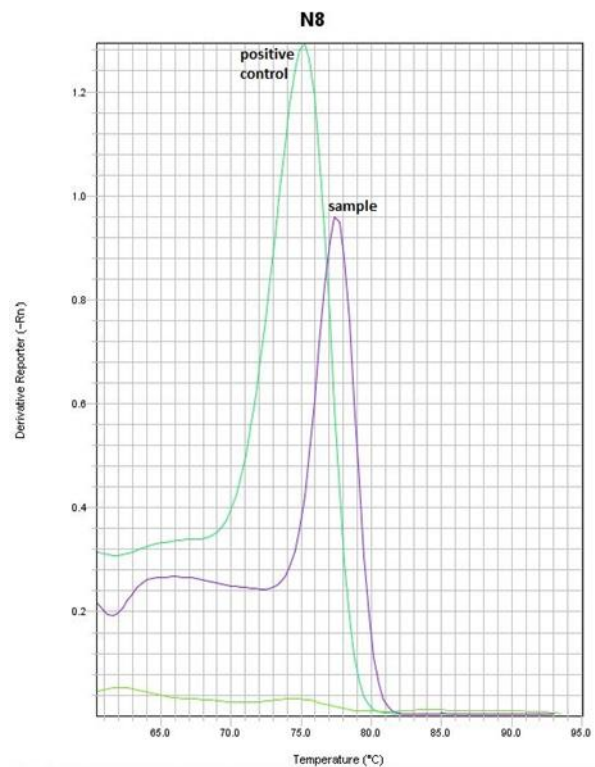
ViroReal® Typing Kit InfA H5N8 is designed for rapid genotyping purposes of previously tested, strong Influenza A positive samples. The specificity for Influenza A H5N8 is ensured by the selection of highly specific primers. The primers have been checked for possible homologies to currently published sequences by sequence comparison analysis. However, the high sequence variability of influenza A has to be kept in mind. We recommend pre-testing of seasonal or local Influenza A strain variants whose virulence has already been confirmed by international agencies. If those subtypes are tested positive with ViroReal® Typing Kit InfA H5N8, high-throughput screening of field samples is indicated.

Influenza A H5 and N8 assays are not prone to generate primer dimers with the supplied master mix.

Interpretation of melt curve data



Applied Biosystems® 7500: 515 nm
Specific melt curve of Influenza A H5-positive sample and positive control, no primer dimer formation



Applied Biosystems® 7500: 515 nm
Specific melt curve of Influenza A N8-positive sample and positive control, no primer dimer formation

For further information on our products, please visit our homepage (www.ingenetix.com)