

Technical Data Sheet:

Quantitative Synthetic Avian Influenza Virus (H5N6) RNA

ATCC® Number	VR-3439SD™
Product Description	Quantitative Synthetic Avian Influenza Virus (H5N6) RNA is a synthetically derived preparation that can be used for assay development, verification, and validation as well as monitoring of day-to-day test variation and lot-to-lot performance of molecular-based assays. The quantitative format allows for the generation of a standard curve for quantitative PCR (qPCR) to determine viral load.
Genetic Target	The synthetic RNA preparation includes two constructs. One construct includes the full genes for the HA and NP regions. The other construct includes the full genes for the NA, M1/M2, and NEP/NS1 regions. This product is based on the A/goose/Guangdong/GS018/2015 influenza virus sequence with few modifications to accommodate manufacturing and product compatibility with H5-specific and N6-specific assays. The section of the hemagglutinin gene encoding the polybasic cleavage site in the protein has been removed.

Publication	Assay Target	Oligo	Sequence (5' to 3')	Number of mismatches with ATCC® VR-3439SD™ based on <i>in silico</i> analysis
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	CCARTRGGKGCKATAAAYTC	0
		Reverse	GTCTGCAGCRTAYCCACTYC	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	GTGACGAATTCATCAATGTRCCG	1
		Reverse	CTCTGGTTTAGTGTTGATGTYCCAA	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TTTATAGAGGGAGGATGG	0
		Reverse	GAGTGGATTCTTTGTCTG	0
		Probe	TGGTAGATGGTTGGTATGGG	0

World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TGGGTACCACCATAGCAATGAGCA	0
		Reverse	AATTCCCTTCCAACGGCCTCAAAC	1
		Probe	TGGGTACGCTGCAGACAAAGAATCCA	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	CARGGGAGTGGDTAYGCBGCAGA	0
		Reverse	ARAAGTTCAGCRTRTARGTCCA	0
		Probe	AARATGAACASTCARTTYGAGG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TACCAAATAYTGCAATTTATTCAAC	0
		Reverse	GTAAYGACCCRTTRGARCACATCC	0
		Probe	CTGGCAATCATDRTGGCTGGTCT	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	CGATCTAAATGGAGTGAAGCCTC	0
		Reverse	CCTTCTCCACTATGTANGACCATTG	1
		Probe	AGCCAYCCAGCTACRCTACA	0
Slomka MJ, et al. Validated H5 Eurasian real-time reverse transcriptase-polymerase chain reaction and its application in H5N1 outbreaks in 2005-2006. Avian Dis 51: 373-377, 2007. PubMed: 17494587	HA	Forward	ACATATGACTACCCACARTATTCAG	1
		Reverse	AGACCAGCTAYCATGATTGC	0
		Probe	TCWACAGTGGCGAGTTCCTAGCA	0
Hoffmann B, et al. Riems influenza a typing array (RITA): An RT-qPCR-based low density array for subtyping avian and mammalian influenza a viruses. Sci Rep 6: 27211, 2016. PubMed: 27256976	HA	Forward	GTTCCCTAGYACTGGCAATCAT	0
		Reverse	AATTCTARATGCAAATTCTGCAYTG	0
		Probe	CTGGTCTATCYTTHTGATGTGYTCCAATG	0
Liu J, et al. Development and application of a triplex real-time PCR assay for the simultaneous detection of avian influenza virus subtype H5, H7 and H9. J Virol Methods 252: 49-56. PubMed: 29129489	HA	Forward	GTACCACCATAGCAATGAGCAG	0
		Reverse	AGTCCAGACATCTAGGAATCCGT	1
		Probe	TACGCTGCAGACAAAGAATCCAC	0
Hassan KE, et al. Improved Subtyping of Avian Influenza Viruses Using an RT-qPCR-Based Low Density Array: 'Riems Influenza a Typing Array', Version 2 (RITA-2). Viruses 14(2): 415, 2022. PubMed: 35216008	HA	Forward 1	GATTYTAARGATTGTAGYGTAGC	0
		Forward 2	GTTCCCTAGYAYTGGCAATCAT	0
		Reverse 1	CTCTCYACCATGTARGACCA	1
		Reverse 2	CTCTCYACTATGTARGACCA	0
		Reverse 3	AATTCTARATGCAAATTCTGCAYTG	0
		Probe 1	CGCACATTGGRTTYCCRAGGAGCC	0
		Probe 2	CTGGTCTATYYTTRTGATGTGCTCC	0

Suwannakarn K, et al. Typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays. J Virol Methods 152(1-2): 25-31, 2008. PubMed: 18598722	HA	Forward	TGGAAAGTGTAAACGGAACGT	0
		Reverse	TGCTAGGGAACGCMCTG	0
		Probe	A+CTC+CA+CTT+AT+TT+CCT+CT+CT	0
Centers for Disease Control and Prevention (U.S.); National Center for Immunization and Respiratory Diseases (U.S.). Influenza Division. Virology Surveillance and Diagnosis Branch. Genomics and Diagnostics Team. Research Use Only CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay Real-Time RT-PCR Primers and Probes. Publish date: July 14, 2020.	M	Forward 1	CAAGACCAATCYGTACCTCTGAC	0
		Forward 2	CAAGACCAATYCTGTACCTYTGAC	0
		Reverse	GCATTYTGGACAAVCGTCTACG	0
		Probe	TGCAGTCCTCGCTCACTGGGCACG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	GACCRATCCTGTACCTCTGAC	0
		Reverse	AGGGCATTYTGGACAAKCGTCTA	0
		Probe	TGCAGTCCTCGCTCACTGGGCACG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	ATGAGYCTTYTAACCGAGGTCGAAACG	0
		Reverse	TGGACAAACGTCTACGCTGCAG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CTTCTAACCGAGGTCGAAACGTA	0
		Reverse	GGTGACAGGATTGGTCTTGTCTTTA	0
		Probe	TCAGGCCCCCTCAAAGCCGAG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CCMAGGTCGAAACGTAYGTTCTCTCTATC	1
		Reverse	TGACAGRATYGGTCTTGTCTTTAGCCAYTCCA	0
		Probe	ATYTGGGCTTTGAGGGGGCCTG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CTTCTAACCGAGGTCGAAACGTA	0
		Reverse	GGTGACAGGATTGGTCTTGTCTTTA	0
		Probe	TCAGGCCCCCTCAAAGCCGAG	0
Spackman E, et al. Development of a Real-Time Reverse Transcriptase PCR Assay for Type A Influenza Virus and the Avian H5 and H7 Hemagglutinin Subtypes. J Clin Microbiol 40(9): 3256-3260, 2022. PubMed: 12202562	M	Forward	AGATGAGTCTTCTAACCGAGGTCG	0
		Reverse	TGCAAAAACATCTTCAAGTCTCTG	1
		Probe	TCAGGCCCCCTCAAAGCCGA	0
Hoffmann B, et al. Riems influenza a typing array (RITA): An RT-qPCR-based low density array for subtyping avian and mammalian influenza a viruses. Sci Rep 6: 27211, 2016. PubMed: 27256976	M	Forward	AGATGAGTCTTCTAACCGAGGTCG	0
		Reverse	TGCAAAAACATCTTCAAGTYTCTG	0
		Probe	TCAGGCCCCCTCAAAGCCGA	0

Laconi A, et al. Detection of avian influenza virus: a comparative study of the in silico and in vitro performances of current RT-qPCR assays. Sci Rep 10(1): 8441, 2020. PubMed: 32439885	M	Forward	AGATGAGTCTTCTAACCGAGGTCTG	0
		Reverse	TGCAAAACATCTTCAAGTYTCTG	0
		Probe	TCAGGCCCCCTCAAAGCCGA	0
Laconi A, et al. Detection of avian influenza virus: a comparative study of the in silico and in vitro performances of current RT-qPCR assays. Sci Rep 10(1): 8441, 2020. PubMed: 32439885	M	Forward	AGATGAGYCTTCTAACCGAGGTCTG	0
		Reverse	TGCAAAACATCTTCAAGTYTCTG	1
		Probe	TCAGGCCCCCTCAAAGCCGA	0
Laconi A, et al. Detection of avian influenza virus: a comparative study of the in silico and in vitro performances of current RT-qPCR assays. Sci Rep 10(1): 8441, 2020. PubMed: 32439885	M	Forward	GGCCCCCTCAAAGCCGA	0
		Reverse	CGTCTACGYTGAGTCC	0
		Probe	GTGCCCAG	0
Liu J, et al. Development and application of a triplex real-time PCR assay for the simultaneous detection of avian influenza virus subtype H5, H7 and H9. J Virol Methods 252: 49-56, 2018. PubMed: 29129489	M	Forward	GACCAATCCTGTACCTCTGAC	0
		Reverse	GGGCATTTTGGACAAAGCGTCTACG	0
Nagy A, et al. A universal RT-qPCR assay for "One Health" detection of influenza A viruses. PLoS One 16(1): e0244669, 2021. PubMed: 33471840	M	Forward	GGCCCCCTCAAAGCCGA	0
		Reverse	CGTCTACGYTGAGTCC	0
		Probe	TCACTKGGCACGGTGAGCGT	0
Goecke NB, et al. Subtyping of Swine Influenza Viruses Using a High-Throughput Real-Time PCR Platform. Front Cell Infect Microbiol 8: 165, 2018. PubMed: 29872645	M	Forward	CTTCTAACCGAGGTGAAACGTA	0
		Reverse	CACTGGGCACGGTGAGC	0
		Probe	TCAGGCCCCCTCAAAGCCGA	0
Hassan KE, et al. Improved Subtyping of Avian Influenza Viruses Using an RT-qPCR-Based Low Density Array: 'Riems Influenza a Typing Array', Version 2 (RITA-2). Viruses 14(2): 415, 2022. PubMed: 35216008	M	Forward	AGATGAGYCTTCTAACCGAGGTCTG	0
		Reverse 1	TGCAAAAACATCTTCAAGTYTCTG	0
		Reverse 2	TGCAAAIACATCTTCAAGTYTCTG	1
		Probe	TCAGGCCCCCTCAAAGCCGA	0
Leong NKC, et al. A six-plex droplet digital RT-PCR assay for seasonal influenza virus typing, subtyping, and lineage determination. Influenza Other Respir Viruses 14(6): 720-729, 2020. PubMed: 32519796	M	Forward	CTTCTAACCGAGGTGAAACGTA	0
		Reverse	AGGGCATTYTGACAAKCGTCTA	0
		Probe	TCAGGCCCCCTCAAAGCCGAG	0
Suwannakarn K, et al. Typing (A/B) and subtyping (H1/H3/H5) of influenza A viruses by multiplex real-time RT-PCR assays. J Virol Methods 152(1-2): 25-31, 2008. PubMed: 18598722	M	Forward	CATGGARTGGCTAAAGACAAGACC	0
		Reverse	AGGGCATTTTGGACAAKCGTCTA	0
		Probe	ACGCTCA+CCGTGCCAGT	0

Van Elden LJ, et al. Simultaneous detection of influenza viruses A and B using real-time quantitative PCR. J Clin Microbiol 39(1): 196-200, 2001. PubMed: 11136770	M	Forward	GGACTGCAGCGTAGACGCTT	0
		Reverse	CATCCTGTTGTATATGAGGCCCAT	1
		Probe	CATTCTGTTGTATATGAGGCCCAT	0
Ward CL, et al. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. J Clin Virol 29(3): 179-188, 2004. PubMed: 14962787	M	Forward	AAGACCAATCCTGTCACCTCTGA	0
		Reverse	CAAAGCGTCTACGCTGCAGTCC	0
		Probe	TTTGTGTTACGCTCACCGT	0
James J, et al. Proceedings Paper-Avian Diseases 10th AI Symposium Issue Development and Application of Real-Time PCR Assays for Specific Detection of Contemporary Avian Influenza Virus Subtypes N5, N6, N7, N8, and N9. Avian Dis 63(1): 209-218, 2019. PubMed: 31131579	NA	Forward 1	AGGGTGAAAATGAATCCAAATCA	0
		Forward 2	TGAAAATGAATCCAAATCARAAGRTAA	0
		Reverse	AATTCCTATYAGCAGRCTTACYAC	0
		Probe	TGCRTTTCAGCMACAGGARTRACACTATC	0