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Technical Data Sheet:

Quantitative Synthetic Avian Influenza Virus (H5N1) RNA

ATCC® Number	VR-3436SD™
Product Description	Quantitative Synthetic Avian Influenza Virus (H5N1) 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/white-tailed eagle/Japan/OU-1/2022 influenza virus sequence with few modifications to accommodate manufacturing and product compatibility with H5-specific and N1(avian)-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-3436SD™ 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	CCARTRGGKGCKATAAYTC	0
		Reverse	GTCTGCAGCRTAYCCACTYC	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	CCGCAGTATTCAAGAAGAAGC	0
		Reverse	AGACCAGCYAYCATGATTGC	0
		Probe	AGTGCTAGRGAACCTCGCMACTGTAG	1
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TTTATAGAGGGAGGATGG	0
		Reverse	GAGTGGATTCTTGTCTG	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	AATTCCCTCCAACGGCCTCAAAC	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	ARAAGTTCAGCRTTRTARGTCCA	0
		Probe	AARATGAACCASTCARTTYGAGG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TACCAAATAYGTCAATTATTCAAC	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 1	CGATCTAGAYGGGTGAARCCCTC	1
		Forward 2	CGATCTAAATGGAGTGAAGCCTC	1
		Reverse	CCTTCTCCACTATGTANGACCATT	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	ACATATGACTACCCACARTATTAG	1
		Reverse	AGACCAGCTAYCATGATTGC	0
		Probe	TCWACAGTGGCGAGTTCCCTAGCA	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	CTGGTCTATCYTTHTGGATGTGYTCCAATG	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	CTGGTCTATYYTTRTGGATGTGCTCC	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	CAAGACCAATCYTGTCACCTCTGAC	0
		Forward 2	CAAGACCAATYCTGTCACCTYTGAC	0
		Reverse 1	GCATTYTGGACAAAVCGTCTACG	0
		Reverse 2	GCATTTGGATAAAGCGTCTACG	1
		Probe	TGCAGTCCTCGCTCACTGGGCACG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	GACCRATCCTGTCACCTCTGAC	0
		Reverse	AGGGCATTYTGGACAAAKCGTCTA	0
		Probe	TGCAGTCCTCGCTCACTGGGCACG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	ATGAGYCTTYAACCGAGGTCGAAACG	0
		Reverse	TGGACAAANGTCTACGCTGCAG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CTTCTAACCGAGGTCGAAACGTA	0
		Reverse	GGTGACAGGATTGGTCTTGTCTTA	0
		Probe	TCAGGCCCCCTCAAAGCCGAG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CCMAGGTCGAAACGTAYGTTCTCTATC	1
		Reverse	TGACAGRATYGGTCTTGTCTTAGCCAYTCCA	0
		Probe	ATYCGGCTTGAGGGGGCCTG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	CTTCTAACCGAGGTCGAAACGTA	0
		Reverse	GGTGACAGGATTGGTCTTGTCTTA	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	0
		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	AGATGAGTCTTCTAACCGAGGTCG	0
		Reverse	TGCAAARACATCTTCAAGTYTCTG	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	AGATGAGYCTTCTAACCGAGGTCG	0
		Reverse	TGCAAANACATCYTCAAGTCTCTG	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	GGCCCCCTCAAAGCCGA	0
		Reverse	CGTCTACGYTGCAGTCC	0
		Probe	GTGCCAG	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	GACCAATCCTGTCACCTCTGAC	0
		Reverse	GGGCATTTGGACAAAGCGTCTACG	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	CGTCTACGYTGCAGTCC	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	AGATGAGYCTTCTAACCGAGGTCG	0
		Reverse 1	TGCAAAAACATCTTCAAGTYTCTG	0
		Reverse 2	TGCAAAIACATCYTCAAGTYTCTG	0
		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	AGGGCATTYTGGACAAAKCGTCTA	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	AGGGCATTYTGGACAAAKCGTCTA	0
		Probe	ACGCTCA+CCGTGCCAGT	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	TTTGTGTTCACGCTACCGT	0

World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	NA	Forward	GTTTGAGTCTGTTGCTGGTC	0
		Reverse	GCCATTACACATGCACATTCA	0
		Probe	CATGATGGCAYYAGTTGGTTGACAA	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	NA	Forward	AGRCCCTGYTTCTGGGTGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	ATYTGGACYAGTGGGAGCAGCAT	0
Hassan KE, et al. Improved Subtyping of Avian Influenza Viruses Using an RT-qPCR-Based Low Density Array: 'Riets Influenza a Typing Array', Version 2 (RITA-2). Viruses 14(2): 415, 2022. PubMed: 35216008	NA	Forward	GRCCTTGYTTCTGGTKGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	CAATYTGGACYAGTGGGRAGYAGCAT	0
Tsukamoto K, et al. Use of reverse transcriptase PCR to subtype N1 to N9 neuraminidase genes of avian influenza viruses. J Clin Microbiol 47(7): 2301-2303, 2009. PubMed: 19403772	NA	Forward	TCARTCTGYATGRYAA YTGG	1
		Reverse	GGRCARAGAGAKGAATTGCC	0

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