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Technical Data Sheet:

Quantitative Synthetic Avian Influenza Virus (H7N9) RNA

ATCC® Number	VR-3437SD™
Product Description	Quantitative Synthetic Avian Influenza Virus (H7N9) 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/Shanghai/4664T/2013 influenza virus sequence with few modifications to accommodate manufacturing and product compatibility with H7-specific and N9-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-3437SD™ based on *in silico* analysis
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TGTGATGAYGAYTGYATGGCCAG	0
		Reverse	ACATGATGCCCCGAAGCTAAAC	0
		Probe	ATCTGTATTCTATTTTGCATTGCYTC	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TCACAGCAAATACAGGGAAGAG	0
		Reverse	CCCGAAGCTAAACCAGAGTATC	0
		Probe	TGACCCAGTCAAACTAAGCAGCGG	0

World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	CAATCACTGGACCACCTCAA	0
		Reverse	TCACGAATTTCCCAGGATAACA	0
		Probe	TGAGAGGCGAGAAGGAAGTGATGT	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TTTAGCTTCGGGGCATCATGTTT	0
		Reverse	CAAATAGTGCACCGCATGTTTCCA	0
		Probe	TGGGCCTTGTCTTCATATGTGTAAA	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	AGAAATGAAATGGCTCCTGTCAA	0
		Reverse	GGTTTTTTCTTGTATTTTTATATGACTTAG	0
		Probe	AGATAATGCTGCATTCCCGCAGATG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	AACGAACAAACATCCCCA	0
		Reverse	CTTCATTCACGAATTTCCCA	0
		Probe	ACATCACTTCCTTCTCGCCTCT	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	AYAGAATACAGATWGACCCAGT	0
		Reverse	TAGTGCACYGCATGTTTCCA	0
		Probe	TGGTTTAGCTTCGGGGCATCATG	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	GYAGYGGYTACAAAGATGTG	0
		Reverse	GAAGACAAGGCCCATTGCAA	0
		Probe	TGGTTTAGCTTCGGGGCATCATG	0
Inui K, et al. A field-deployable insulated isothermal RT-PCR assay for identification of influenza A (H7N9) shows good performance in the laboratory. Influenza Other Respir Viruses 13(6): 610-617, 2019. PubMed: 31487118	HA	Forward	GCTTCGGGGCATCATGTT	0
		Reverse	GCACCGCATGTTTCCATTCTT	0
		Probe	ATTGCAATGGGCCTTGTC	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	CCATTRCAATGGCTAGAAG	0
		Reverse	AATAGAATACAGATWGACCCAGT	0
		Probe	CATGATGCCCCGAAGCTAAACC	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	CAACTGAAACRGTRGARCG	0
		Reverse 1	CAGGAGYCCACATTGACC	0
		Reverse 2	TTCTAGGAATTGGTCACATTG	0
		Probe	CCCAGGATYTGCTCAARAGGRAAAA	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	GCATTYTGGACAAAVCGTCTACG	0
		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	ATGAGYCTTYTAACCGAGGTCGAAACG	0
		Reverse	TGGACAAANCGTCTACGCTGCAG	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	ATYTCGGCTTTGAGGGGGCCTG	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	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	AGATGAGYCTTCTAACCGAGGTCG	0
		Reverse	TGCAAANACATCYTCAAGTCTCTG	0
		Probe	TCAGGCCCCCTCAAAGCCGA	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	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	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	CTTCTAACCGAGGTCGAAACGTA	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	TGCAAAAACATCTTCAAGTYTCTG	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	CTTCTAACCGAGGTCGAAACGTA	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	AGGGCATTTTGGACAAAKCGTCTA	0
		Probe	ACGCTCA+CCGTGCCCAGT	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	TTTGTGTTCACGCTCACCGT	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	NA	Forward	GGAGTGGTTACAGTGGATCTTT	0
		Reverse	CTTTATCCTCCTTGGGTCTTCC	0
		Probe	AAACACGCTCGATAGCAGTCCC	0

World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	NA	Forward	TAGCAATGACACACACTAGTCAAT	0
		Reverse	ATTACCTGGATAAGGGTCATTACACT	0
		Probe	AGACAATCCCCGACCGAATGACCC	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	NA	Forward	CTCATTGGAATGGCAAACCT	0
		Reverse	TTCCATTTGGATGTTGGTGA	0
		Probe	TGCAATTGCTCACACTCACA	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	AGYATAGTATCRATGTGTTCCAG	0
		Reverse	AAGTACTCTATTTTAGCCCCATC	0
		Probe	TTCCTBGGACAATGGAACTGGCC	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	AGYATAGTATCRATGTGTTCCAG	0
		Reverse	AAGTACTCTATTYTAGCCCCRTC	0
		Probe	TTCCTBGGACAATGGAACTGGCC	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	NA	Forward	AGYATAGTATCRATGTGTTCCAG	0
		Reverse	TTCCTRGGACAATGGRACTGGCC	0
		Probe	GTACTCTATTYTAGCCCCRTC	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	GTAATAGGCACRATYGCAGT	0
		Reverse	CCTTTRGTYARRTTATTGAA	0