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Technical Data Sheet:

Quantitative Synthetic Influenza A Virus (H1N1)pdm09 RNA

ATCC® Number	VR-3388SD™
Product Description	Quantitative Synthetic Influenza A virus (H1N1)pdm09 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/Netherlands/2629/2009 (H1N1)pdm09 influenza virus sequence with few modifications to accommodate manufacturing and product compatibility with diagnostically relevant assays.

Publication	Assay Target	Oligo	Sequence (5' to 3')	Number of mismatches with ATCC® VR-3388SD™ based on *in silico* analysis
Shu B, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. J Clin Microbiol 49(7): 2614-2619, 2011. PubMed: 21593260	HA	Forward	GTGCTATAAACACCAGCCTYCCA	0
		Reverse	CGGGATATTCCTTAATCCTGTRGC	0
		Probe	CAGAATATACAtCCRGTCACAATTGGARAA	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	TGAGCTCAGTGTCATCATTTGA	0
		Reverse	TGCTGAGCTTTGGGTATGAA	0

World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	GACAAAATAACAAACGAAGCAACTGG	0
		Reverse	GGGAGGCTGGTGTTTATAGCACC	0
		Probe	GCATTCGCAATGGAAAGAAATGCTGG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	AAACTATGCAAACTAAGAGGGGT	0
		Reverse	TGTTTCCACAATGTAGGACCA	0
		Probe	CCAGAGTGTGAATCACTCTCCACA	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	AGAAAAGAATGTAACAGTAACACACTCTGT	0
		Reverse	GTTTCCACAATGTAGGACCATG	0
		Probe	CAGCCAGCAATGTTGCATTTACC	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	HA	Forward	AGAAAAGAATGTAACAGTAACACACTCTGT	0
		Reverse	TGTTTCCACAATGTARGACCAT	0
		Probe	CAGCCAGCAATRTTRCATTTACC	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	ACACAATATGTATAGGYTAHCATGC	1
		Reverse	GAGTGTGTYACTGTYACATTCTT	0
		Probe	TCDACMGACACTGTWGACACAGTACTNGA	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 1	AGGAATGTCCCRTCYATTCAATC	1
		Forward 2	CCCGTCYATTCAATCYAGAGG	0
		Reverse 1	GGTGATAACCRTACCANCCATC	0
		Reverse 2	TCATTTTGATGRTGATAACCRTACCA	0
		Probe	CATYCCWGTCCAYCCYCCTTCAATGAA	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	HA	Forward	AGTTCAAGCCGGAAATAGCA	0
		Reverse	CCCGGCTCTACTAGTGTCCA	0
		Probe	CCCAAAGTGAGGRATCAAGAAGGGAG	0
Haach V, et al. One-step multiplex RT-qPCR for the detection and subtyping of influenza A virus in swine in Brazil. J Virol Methods 269: 43-48, 2019. PubMed: 30959063	HA	Forward	CACAAAWTTGAGACTGGYMACA	0
		Reverse	CTGTCCAYCCYCCTTCAAT	0
		Probe	CCTATTTGGRGCCATTGCYGGTT	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	HA	Forward	GTGCTATAAACACCAGCCTCCCA	0
		Reverse	AGAYGGGACATTCCTCAATCCTG	1
		Probe	ATGTAAAAAGCACAAAATTGAGACTGGCCA	0
Selvaraju SB, Selvarangan R. Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. J Clin Microbiol 48(11): 3870-3875, 2010. PubMed: 20844230	HA	Forward	AAGCAACAAAAATGRAGGCAATACTA	0
		Reverse	TCTGTTGAATTGTTCGCATGATAA	0
		Probe	TTRCAACCGCAAATGCAGACACATTATG	0
Shu B, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. J Clin Microbiol 49(7): 2614-2619, 2011. PubMed: 21593260	NP	Forward	GCACGGTCAGCACTTATYCTRAG	1
		Reverse	GTGRGCTGGGTTTTCATTTGGTC	0
		Probe	CYACTGCAAGCCCAtACACACAAGCAGGCA	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	NP	Forward	ACGGTCAGCACTCATTCTG	0
		Reverse	ACCAGTGAGTACCCTTCC	0
		Probe	TCATGCCCACTTGCTACTGCAAGC	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	1
		Reverse 1	GCATTYTGGACAAAVCGTCTACG	0
		Reverse 2	GCATTTTGGATAAAGCGTCTACG	1
		Probe	TGCAGTCCTCGCTCACTGGGCACG	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	M	Forward	GACCRATCCTGTCACCTCTGAC	1
		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	1
		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	1
		Probe	ATYTCGGCTTTGAGGGGGCCTG	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	TGCAAAGACACTTTCCAGTCTCTG	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	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	GACCAATCCTGTCACCTCTGAC	1
		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
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	CTGGCTAGCACTACRGCA	0
		Reverse	TACCATYTGCCTAGTCTGATTA	0
		Probe	CTCYATGGCCTCTGCTGCCTGT	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	TGCAAAGACACTTTCCAGTCTCTG	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	ACGC+TCACCG+TGCCC+AGT	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	1
		Reverse	CAAAGCGTCTACGCTGCAGTCC	0
		Probe	TTTGTGTTCACGCTCACCGT	0
World Health Organization. WHO information for the molecular detection of influenza viruses. Publish date: February 2021.	NA	Forward	AGACCTTGCTTCTGGGTTGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	ATCTGGACTAGCGGGAGCAGCAT	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	AGRCCTTGYTTCTGGGTTGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	ATYTGGACYAGTGGGAGCAGCAT	1
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	NA	Forward	CCTTGCTTCTGGGTTGAACTAATC	0
		Reverse	AGTGTCACTATTTACACCACAAAAGG	1
		Probe	TGCTCCCGCTAGTCCAGATTGTGTTCTCTT	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	NA	Forward	AGRCCTTGYTTCTGGGTTGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	ATYTGGACYAGTGGGAGCAGCAT	1
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	NA	Forward	CGAAATGAGTGCCCCTAATTATC	1
		Reverse	CGATTCGAGCCATGCCAGTTA	0
		Probe	+C+CT+GATTCT+AGTGAAATCA+C	0
Haach V, et al. One-step multiplex RT-qPCR for the detection and subtyping of influenza A virus in swine in Brazil. J Virol Methods 269: 43-48, 2019. PubMed: 30959063	NA	Forward	GAGGARTGYTCYTGYTATCCTGA	0
		Reverse	AAAGACACCCAHGGYCGRTT	0
		Probe	ATGTGTRTGCAGGGATAACTGGCATGG	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	GRCCTTGYTTCTGGGTKGA	0
		Reverse	ACCGTCTGGCCAAGACCA	0
		Probe	CAATYTGGACYAGTGGRAGYAGCAT	1

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	TCARTCTGYATGRYAAYTGG	1
		Reverse	GGRCARAGAGAKGAATTGCC	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	NS1	Forward	GAGGAAATGTCACGAGACTG	0
		Reverse	ACTGAAGTTCGCTTTCAGTAC	0
		Probe	TTCCATGACCGCCTGGTCCAATCG	0
Rönkkö E, et al. Validation and diagnostic application of NS and HA gene-specific real-time reverse transcription-PCR assays for detection of 2009 pandemic influenza A (H1N1) viruses in clinical specimens. J Clin Microbiol 49(5): 2009-2011, 2011. PubMed: 21367994	NS1	Forward	GCGATTTGCAGACAATGGATTG	0
		Reverse	CTGTTTCGATATCGAGGCCA	0
		Probe	AAAGTCCTTAAAAGGAAGAGGCAACACC	0
Rönkkö E, et al. Validation and diagnostic application of NS and HA gene-specific real-time reverse transcription-PCR assays for detection of 2009 pandemic influenza A (H1N1) viruses in clinical specimens. J Clin Microbiol 49(5): 2009-2011, 2011. PubMed: 21367994	NS1	Forward	GAAACAAATCGTGGAATGGATCT	0
		Reverse	TCGTGACATTTCCTCGAGG	0
		Probe	CAATTGCATCTGTACCTACTTCGCGC	1
Rönkkö E, et al. Validation and diagnostic application of NS and HA gene-specific real-time reverse transcription-PCR assays for detection of 2009 pandemic influenza A (H1N1) viruses in clinical specimens. J Clin Microbiol 49(5): 2009-2011, 2011. PubMed: 21367994	NS1	Forward	AGACCTTCACTACCTCCAGAG	0
		Reverse	TTTCTTCAATTAACCACCTTATTTCC	0
		Probe	TGAAAAGTGGCGAGAGCAATTGGGACA	1