

Swine Influenza A (H1N1) Real-Time RT-PCR Assay

Catalog No. NR-15577

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Contributor and Manufacturer:

BEI Resources

Product Description:

The Swine Influenza A (H1N1) Real-Time RT-PCR Assay includes oligonucleotide primers and dual-labeled hydrolysis (TaqMan[®]) probes to be used in real-time RT-PCR assays for the *in vitro* qualitative detection and characterization of swine influenza viruses. The TaqMan[®] probes are labeled with the reporter molecule 6-carboxyfluorescein (6-FAM) and with the quencher, Black Hole Quencher™ 1 (BHQ1).

NR-15577 contains the following components: 1) A Universal Influenza A primer and probe set designed to detect type A influenza viruses, 2) A Swine Influenza A primer and probe set designed to detect swine influenza A viruses, 3) A Swine Influenza H1 primer and probe set designed to detect swine H1 influenza A viruses, 4) A Human RNase P Positive Control primer and probe set designed to detect the presence of RNA from the human RNase P gene in human samples, indicating acceptable sample quality, 5) A Swine Influenza A (H1N1) Positive Control containing PBS spiked with beta-propiolactone (BPL)-inactivated swine H1N1 influenza A virus and human epithelial cells, 6) A Novel Influenza A (H1N1) Positive Control containing PBS spiked with BPL-inactivated novel H1N1 influenza A virus and human epithelial cells, and 7) A Human Specimen Extraction Control containing PBS spiked with human epithelial cells.

NR-15577 contains enough primer, probe and control material for approximately 1000 tests. See Appendix I for assay information.

Material Provided:

Primers are provided lyophilized at approximately 20 nmoles per vial. Probes are provided lyophilized at approximately 5 nmoles per vial. Primers and probes should be reconstituted with 500 µL of nuclease-free water. The Positive Controls and the Extraction Control are provided in 500 µL volumes.

Packaging/Storage:

NR-15577 should be stored at -20°C. Reconstituted aliquots of primers and probes should be stored at -20°C or colder and may be stored frozen for up to 6 months. Thawed aliquots of probes and primers may be stored in the dark up to 1 month at 2°C to 8°C.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Swine Influenza A (H1N1) Real-Time RT-PCR Assay, NR-15577."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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**APPENDIX I
Swine Influenza A (H1N1) Real Time RT-PCR Assay**

Recommended Reagents/Equipment

BEI Resources

Reagent/Equipment	Source	Catalog #
Universal Influenza A Probe	BEI Resources	NR-15578
Universal Influenza A Forward Primer	BEI Resources	NR-15579
Universal Influenza A Reverse Primer	BEI Resources	NR-15580
Swine Influenza A Probe	BEI Resources	NR-15581
Swine Influenza A Forward Primer	BEI Resources	NR-15582
Swine Influenza A Reverse Primer	BEI Resources	NR-15583
Swine Influenza H1 Probe	BEI Resources	NR-15584
Swine Influenza H1 Forward Primer	BEI Resources	NR-15585
Swine Influenza H1 Reverse Primer	BEI Resources	NR-15586
Human RNase P Positive Control Probe	BEI Resources	NR-15587
Human RNase P Positive Control Forward Primer	BEI Resources	NR-15588
Human RNase P Positive Control Reverse Primer	BEI Resources	NR-15589
Swine Influenza A (H1N1) Positive Control	BEI Resources	NR-15590
Novel H1N1 2009 (Swine) Influenza A Positive Control	BEI Resources	NR-15627
Human Specimen Extraction Control	BEI Resources	NR-15591

Other Vendors

QIAamp [®] Viral RNA Mini Kit or equivalent	QIAGEN [®]	52904
SuperScript [®] III Platinum [®] One-Step qRT-PCR Kit or equivalent	Invitrogen [™]	11732-020
Nuclease-Free Water	Promega	P1193
iQ [™] 5 Real-Time PCR Detection System or equivalent	Bio-Rad	-----

Reaction Mix¹

Reagent	Stock Concentration	Volume per Reaction (µL)
Nuclease-Free Water	---	5.5
2X Reaction Mix	2X	12.5
Forward Primer	40 µM	0.5
Reverse Primer	40 µM	0.5
Probe ²	10 µM	0.5
SuperScript [™] III RT/Platinum [®] Taq Mix	---	0.5
Nucleic acid sample ³	---	5
		Total – 25 µL

¹Reaction mix should be kept in bench-top cooler until ready for use.

²6-carboxyfluorescein (6-FAM) probe must be protected from light at all times.

³Nucleic acids extracted from NR-15590, NR-15591 and NR-15627 should be diluted 1:10 prior to use as template in the assay.

Cycling Protocol

	Cycle	# of Repeats	Step	Temperature	Temperature
Reverse Transcription	1	1	1	50°C	30 minutes
<i>Taq</i> Inhibitor Activation	2	1	1	95°C	2 minutes
Denature Anneal ¹	3	45	1 2	95°C 55°C	15 seconds 30 seconds

¹Fluorescence data should be collected during the annealing step.

Instructions

1. Extract nucleic acid from the Swine Influenza A (H1N1) Positive Control (NR-15590), the Novel H1N1 2009 (Swine) Influenza A Positive Control (NR-15627), the Human Specimen Extraction Control (NR-15591) and the test samples. Extracted material from NR-15590, NR-15627 and NR-15591 should be diluted 1:10 prior to use as template in the assay.
2. Prepare working stock aliquots of the primers and probes at concentrations of 40 µM (primers) and 10 µM (probes).
3. A No Template Control and nucleic acid extracted from the Swine Influenza A (H1N1) Positive Control (NR-15590) and the Novel H1N1 2009 (Swine) Influenza A Positive Control (NR-15627) should be included in each run for all primer and probe sets.
4. Nucleic acid extracted from the Human Specimen Extraction Control (NR-15591) should be used with the Human RNase P Positive Control primer and probe set as an additional control. This control validates the nucleic acid extraction procedure and reagent integrity for unknown human samples.