

Influenza Virus Real-Time RT-PCR Assay

Catalog No. NR-15592

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Contributor and Manufacturer:

BEI Resources

Product Description:

The Influenza Virus Real-Time RT-PCR 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 seasonal (A/H1, A/H3 and B) and A/H5 (asian-lineage) 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-15592 contains the following components: 1) an influenza A primer and probe set designed to detect type A influenza viruses, 2) an influenza H1 primer and probe set designed to detect H1 influenza A viruses, 3) an influenza H3 primer and probe set designed to detect H3 influenza A viruses, 4) an influenza H5a primer and probe set designed to detect H5a influenza A viruses, 5) an influenza H5b primer and probe set designed to detect H5b influenza A viruses, 6) an influenza B primer and probe set designed to detect influenza B viruses, 7) 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, 8) a Human Seasonal Influenza Virus Positive Control containing PBS spiked with beta-propiolactone (BPL)-inactivated seasonal influenza viruses (A/H1, A/H3 and B) and human epithelial cells, 9) an Influenza A/H5 (Asian-Lineage) Positive Control containing PBS spiked with formalin-inactivated, detergent-disrupted and genetically modified reassortant influenza virus (A/H5) and human epithelial cells, and 10) a Human Specimen Extraction Control containing PBS spiked with human epithelial cells.

NR-15592 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-15592 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: Influenza Virus Real-Time RT-PCR Assay, NR-15592."

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.

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**APPENDIX I
Influenza Virus Real Time RT-PCR Assay**

Recommended Reagents/Equipment

BEI Resources

Reagent/Equipment	Source	Catalog #
Influenza A Probe	BEI Resources	NR-15593
Influenza A Forward Primer	BEI Resources	NR-15594
Influenza A Reverse Primer	BEI Resources	NR-15595
Influenza H1 Probe	BEI Resources	NR-15596
Influenza H1 Forward Primer	BEI Resources	NR-15597
Influenza H1 Reverse Primer	BEI Resources	NR-15598
Influenza H3 Probe	BEI Resources	NR-15599
Influenza H3 Forward Primer	BEI Resources	NR-15600
Influenza H3 Reverse Primer	BEI Resources	NR-15601
Influenza H5a Probe	BEI Resources	NR-15602
Influenza H5a Forward Primer	BEI Resources	NR-15603
Influenza H5a Reverse Primer	BEI Resources	NR-15604
Influenza H5b Probe	BEI Resources	NR-15605
Influenza H5b Forward Primer	BEI Resources	NR-15606
Influenza H5b Reverse Primer	BEI Resources	NR-15607
Influenza B Probe	BEI Resources	NR-15608
Influenza B Forward Primer	BEI Resources	NR-15609
Influenza B Reverse Primer	BEI Resources	NR-15610
Human RNase P Positive Control Probe	BEI Resources	NR-15611
Human RNase P Positive Control Forward Primer	BEI Resources	NR-15612
Human RNase P Positive Control Reverse Primer	BEI Resources	NR-15613
Human Seasonal Influenza Virus Positive Control	BEI Resources	NR-15614
Influenza A/H5 (Asian-Lineage) Positive Control	BEI Resources	NR-15615
Human Specimen Extraction Control	BEI Resources	NR-15616

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

¹All reaction mix material should be kept ice cold until ready for use.

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

³Nucleic acids extracted from NR-15614 and NR-15615 should be diluted 1:10 prior to use as template in the assay. **NR-15616 should be used undiluted in the assay.**

Cycling Protocol

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

¹Fluorescence data should be collected during the annealing step.

Instructions

1. Extract nucleic acid from the Human Seasonal Influenza Virus Positive Control (NR-15614), the Influenza A/H5 (Asian-Lineage) Positive Control (NR-15615), the Human Specimen Extraction Control (NR-15616) and the test samples. Extracted material from NR-15614 and NR-15615 should be diluted 1:10 prior to use as template in the assay. Extracted material from NR-15616 should be used undiluted 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 Human Seasonal Influenza Virus Positive Control (NR-15614), the Influenza A/H5 (Asian-Lineage) Positive Control (NR-15615) should be included in each run for all primer and probe sets.
4. Nucleic acid extracted from the Human Specimen Extraction Control (NR-15616) 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.
5. Each signature (e.g. Influenza A, Influenza B, H1, H3, H5a, H5b and RNase P) should be analyzed independently and threshold values determined manually to best address the performance profiles of each primer and probe set.
6. We have observed some limited cross reactivity (threshold cycles ≤ 37) between the H1 primer and probe set and the Influenza A/H5 (Asian-Lineage) Positive Control material (NR-15615).