SUPPORTING INFECTIOUS DISEASE RESEARCH

Influenza A Virus H3 Primers

Catalog No. NR-12075

This reagent is the tangible property of the U.S. Government.

For research use only. Not for human use.

Contributor:

BEI Resources

Manufacturer:

Integrated DNA Technologies, Inc.

Product Description:

Influenza A viruses are classified into subtypes and named based on the identity of their neuraminidase and hemagglutinin (HA) surface proteins. NR-12075 contains forward and reverse primers that specifically amplify a region of the HA gene of influenza A virus subtype 3 (H3). A protocol is outlined in Appendix I.

Material Provided:

Each vial contains approximately 80 μ L of a mixture of forward and reverse primers in TE buffer (pH 7.0). The concentration is shown on the Certificate of Analysis.

Packaging/Storage:

Primers were packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C or colder upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Influenza A Virus H3 Primers, NR-12075."

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. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see <u>www.cdc.gov/biosafety/publications/bmbl5/index.htm</u>.

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References:

 Lee, M. S., et al. "Identification and Subtyping of Avian Influenza Viruses by Reverse Transcription-PCR." <u>J.</u> <u>Virol. Methods</u> 97 (2001): 13-22. PubMed: 11483213.

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APPENDIX I

Influenza A Virus H3 Primers

Recommended Reagents/Equipment

Reagent	Source	Catalog #
Influenza A Virus H3 Primers	BEI Resources	NR -12075
Positive Control Template, Genomic RNA from Influenza A Virus, A/Aichi/2/1968 (H3N2)	BEI Resources	Genomic RNA extracted from NR-3177
Qiagen OneStep RT-PCR Kit	Qiagen	210212

Reaction Mix¹

Reagent	Stock Concentration	Volume per Reaction (µL)
RNase-free water		19
Qiagen OneStep RT-PCR Buffer	5X	10
Q Solution	5X	10
dNTP Mix	10 mM each	2
Qiagen OneStep RT-PCR Enzyme Mix		2
Primers ²	25 µM (each primer)	2
Template	50 to 5000 ng	5
		Total – 50 μL

¹Reaction mix should be kept on bench-top cooler until ready for use. ²Primers are supplied at working stock concentrations.

Cycling Protocol

Cycle	# of Repeats	Step	Conditions	
1	1	1	50°C for 30 minutes	
1	1	1	95°C for 15 minutes	
2	45	1	94°C for 30 seconds	
		2	53°C for 30 seconds	
		3	72°C for 1 minute	
3	1	1	72°C for 1 minute	
4	Indefinite	1	Hold at 4°C	