

Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb)

Catalog No. NR-58622

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Product Description:

Monkeypox virus, hMPXV/USA/MA001/2022 was isolated from a human in Massachusetts, USA in May 2022, during an epidemic of mpox. NR-58622 lot 70054242 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (BSC-40; ATCC® CRL-2761™) with the deposited material and incubating in Eagle's Minimum Essential Medium (EMEM; HyClone) supplemented with 2% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin solution for 4 days at 37°C. The harvested cells were freeze/thawed three times and recombined with the spin-clarified supernatant.

Passage History:

B(1)/B(1) (Centers for Disease Control and Prevention/The University of Texas Medical Branch); B = BSC-40 cells

Lot: 70054242

Manufacturing Date: 15JUL2022

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in BSC-40 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hMPXV/USA/MA001/2022 (GenBank: ON563414.3)	99.99% identity with isolate hMPXV/USA/MA001/2022 (GenBank: ON563414.3)
Titer by TCID₅₀ Assay in BSC-40 Cells by Cytopathic Effect¹	Report results	1.8 × 10 ⁶ TCID ₅₀ per mL
Sterility (14-day incubation) Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud agar, 37°C and 26°C, aerobic Trypticase Soy agar, 37°C, aerobic Trypticase Soy agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C, aerobic	No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination MycoSensor QPCR Assay Kit	Negative	Negative

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

/Sonia Bjorum Brower/
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06 MAR 2023

Technical Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-58622 lot 70054242

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of four SNPs, one insertion (INS) and two deletions (DEL) when compared to GenBank ON563414.3 (see Table I below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-58622 lot 70054242 and reference sequence GenBank ON563414.3

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
INS	G28540insA	193	1	5.6995%	MPXVgp032	Amino acids148-149 MN → amino acids 148-150 NEL
SNP	G34325A	69	1	68.1159%	MPXVgp041	S128L
SNP	C55125T	86	1	32.5581%	MPXVgp059	A12T
SNP	G104962A	43	1	6.9767%	MPXVgp108	Silent mutation
SNP	G119451A	170	1	63.5294%	MPXVgp121	L52F
DEL	Δ136523-136525	129	-3	6.9767%	MPXVgp138	ΔD (amino acid 385)
DEL	Δ173267-173268	42	-2	7.1429%	Intergenic	Untranslated