

Certificate of Analysis for NR-4076

pCR®II-TOPO® Containing Hemagglutinin (HA) Gene from Monkeypox Virus, Zaire 79, Linearized

Catalog No. NR-4076

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

Product Description: The hemagglutinin (HA) gene from monkeypox virus, Zaire 79 (BEI Resources NR-2324) was amplified from extracted DNA and cloned into the phagemid vector, pCR®II-TOPO®. The plasmid was produced in *Escherichia coli* DH5 α TM-T1^R cells, extracted using a QIAGEN Plasmid Maxi Kit and linearized with *Hin*dIII. The sequence of the HA gene of NR-4076 differs from the monkeypox virus, Zaire 79 HA sequence (GenBank: DQ011155) by one nucleotide at position 516 (T to C).

Lot¹: 7662479 Manufacturing Date: 25AUG2006

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Linearized Plasmid DNA ²	Migrates as a single band at ~ 5,100 bp	Migrates as a single band at ~ 5,100 bp
Sequencing of HA Gene (942 bp)	Report results	One nucleotide difference from GenBank DQ011155 at position 516 of the HA gene (T to C)
DNA Content by PicoGreen® Measurement	Report results	1.37 µg per mL (0.14 µg/mL)
Concentration of DNA Molecules	Calculated using PicoGreen® concentration and molecular weight of plasmid	2.45 X 10 ¹¹ molecules per mL (1.225 X 10 ⁹ molecules per 5 μL)

¹DNA from monkeypox virus, Zaire 79 (BEI Resources NR-2324, lot 4729797) was extracted using a QIAamp Viral RNA Minikit (QIAGEN 52904). The amplified HA gene was cloned into the pCR®II-TOPO® plasmid vector using TOPO TA Cloning® Kit Dual Promoter (Invitrogen™ K4620). Plasmid DNA was extracted using a Plasmid Maxi Kit (QIAGEN 12162).

²Purified plasmid was linearized with *Hin*dIII (New England BioLabs, Inc. R0105S).

Date: 27 FEB 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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