

**Plasmid pETMPOX/L1RoΔTM Containing the M1R Gene from Monkeypox Virus, Zaire 79**

**Catalog No. NR-3023**

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

**Product Description:** The monkeypox M1R gene (GenBank AY160187) was amplified from the plasmid pMPOX/L1Ro by PCR and subcloned into the prokaryotic expression vector pET26b (Novagen®), which contains coding sequences for a 6-histidine tag at the C-terminus of the expressed protein. The transmembrane (TM) domain of the M1R gene was deleted during subcloning so that the resulting vector would express only the extracellular domain of the protein. The plasmid was produced in *Escherichia coli* (*E. coli*) DH5α™ cells and extracted using a QIAGEN® EndoFree® Plasmid Maxi Kit.

**Lot<sup>1</sup>: 59149139**

**Manufacturing Date: 17JUN2010**

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Restriction Enzyme-digested Plasmid DNA <sup>2</sup>	Two bands observed at ~ 5,250 bp and ~ 550 bp	Two bands observed at ~ 5,250 bp and ~ 550 bp
Sequencing of M1R Open Reading Frame (549 nucleotides)	Report results	Insert is intact and in frame; M1R codons 4 – 186 are identical to GenBank AY160187 <sup>3</sup>
DNA Concentration and Content by PicoGreen® Measurement	Report results	0.3 ng per µL (30 ng per vial)

<sup>1</sup>Plasmid DNA was extracted using an EndoFree® Plasmid Maxi Kit (QIAGEN® 12362).

<sup>2</sup>Purified plasmid was digested with *Nde*I (New England BioLabs® Inc. R0111L) and *Not*I (New England BioLab® Inc. R0189L).

<sup>3</sup>The first three codons of the M1R ORF were replaced with a single initiator methionine codon during the PCR subcloning procedure. See Heraud, J. M., et al. "Subunit Recombinant Vaccine Protects Against Monkeypox." *J. Immunol.* 177 (2006): 2552-2564 (PubMed: 16888017). Note also that codons 187 – 250 (including the transmembrane domain) were deleted and replaced with vector-derived sequences.

**Date:** 13 DEC 2011

**Signature:**



**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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

