

Plasmid pETMPOX/A27Lo Containing the A29L Gene from Monkeypox Virus, Zaire 79

Catalog No. NR-3022

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

Product Description: The monkeypox A29L gene (GenBank AY160186) was amplified from plasmid pMPOX/A27Lo 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 resulting bacterial expression plasmid, pETMPOX/A27Lo, was produced in *Escherichia coli* (*E. coli*) DH5α™ cells and extracted using a QIAGEN® EndoFree® Plasmid Maxi Kit.

Lot¹: 59149138

Manufacturing Date: 17JUN2010

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Restriction Enzyme-Digested Plasmid DNA²	Two bands observed at ~ 5,250 bp and ~ 350 bp	Two bands observed at ~ 5,250 bp and ~ 350 bp
Sequencing of A29L Open Reading Frame (330 nucleotides)	Report results	Insert is intact and in frame with a single nucleotide difference from GenBank AY160186 at position 324 of the A29L gene (A to C) ³
DNA Concentration and Content by PicoGreen® Measurement	Report results	0.4 ng per µL (40 ng per vial)

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

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

³This silent nucleotide change was introduced by the reverse primer used in 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 this reference contains a typographical error in the sequence of the forward primer; the actual sequence of the insert is identical to AY160186 in that region.

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.

