

Plasmid pETMPOX/A33RoΔTM Containing the A35R Gene from Monkeypox Virus, Zaire 79

Catalog No. NR-3024

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Product Description: The monkeypox A35R gene (GenBank AY160188) was amplified from the plasmid pMPOX/A33Ro 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 A35R 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.

Lot¹: 59149140

Manufacturing Date: 15JUL2010

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Restriction Enzyme-digested Plasmid DNA ²	Two bands observed at ~ 5,250 bp and ~ 400 bp	Two bands observed at ~ 5,250 bp and ~ 400 bp
Sequencing of A35R Open Reading Frame (366 nucleotides)	Report results	Insert is intact and in frame; A35R codons 60 – 181 are identical to GenBank AY160188 ³
DNA Concentration and Content by PicoGreen® Measurement	Report results	0.2 ng per µL (20 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).

³The first 59 codons of the A35R ORF (including the transmembrane domain) 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).

Date: 13 DEC 2011

Signature:



Title:

Technical Manager, BEI Authentication or designee

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