b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-3025

Plasmid pETMPOX/B5RoΔTM Containing the B6R Gene from Monkeypox Virus, Zaire 79

Catalog No. NR-3025

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

Product Description: The monkeypox B6R gene (GenBank AY160189) was amplified from the plasmid pMPOX/B5Ro 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.[1] The transmembrane (TM) domain of the B6R 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[1]: 59149141 **Manufacturing Date: 15JUL2010**

TEST	SPECIFICATIONS	RESULTS
Agarose Gel Electrophoresis of Restriction Enzyme-digested Plasmid DNA[2]	Two bands observed at ~ 5,250 bp and ~ 850 bp	Two bands observed at ~ 5,250 bp and ~ 850 bp
Sequencing of B6R Open Reading Frame (837 nucleotides)	Report results	Insert is intact and in frame; B6R codons 1 – 279 are identical to GenBank AY160189[3]
DNA Concentration and Content by PicoGreen® Measurement	Report results	0.3 ng per µL (30 ng per vial)

[1]Plasmid DNA was extracted using an EndoFree® Plasmid Maxi Kit (QIAGEN® 12362).
[2]Purified plasmid was digested with *Nde*I (New England BioLabs, Inc. R0111L) and *Not*I (New England BioLabs, Inc. R0189L).
[3]The final 38 codons of the B6R ORF (including the transmembrane domain) were deleted 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:** Dorothy C. Young

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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