

Certificate of Analysis for NR-19392

Plasmid pMRLB76 Containing Gene ML2028 (Protein Ag85B) from Mycobacterium leprae

Catalog No. NR-19392

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

Product Description: NR-19392 is a recombinant expression vector containing *Mycobacterium leprae* gene ML2028, which encodes the antigen 85B protein [also known as fibronectin-binding protein B (FbpB)] that is involved in cell wall mycoloylation. Gene ML2028 was amplified by PCR and cloned into pET28b for expression in *Escherichia coli*, strain TOP10. The expressed protein is histidine-tagged and has an observed molecular weight of 34.8 kDa.

Lot: 61002001 Manufacturing Date: 12MAY2011

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for the bulk lot pMRLB76 02.04.2011 is attached. This lot was received as dry pellets from CSU and rehydrated in TE buffer (10 mM Tris-HCl, 1 mM EDTA buffer, pH 8) at ATCC $^{\otimes}$. Approximately 50 µL of a 0.02 µg/µL solution was dispensed per vial yielding a final amount of 1 µg per vial. A plasmid map is attached.

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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-19392

Recombinant Plasmid Quality Control Record

Plasmid designation

pMRLB76 (pET28-Ag85B ML2028)

Lot Number

pMRLB76 02.04.2011

Notebook/Pgs Notebook detail JNKBook#1/ pp.126-127, 131 Plasmid Prep pp. 126-127 A260 and stock dilution pp.126

QC gel pp. 131

Media used

LB Broth 50 µg/mL Kanamycin

Culture size

1 x 1L

Growth conditions:

Temp 37°C

Time 16 hr.

Shaker speed 130 rpm

Plasmid prep type (mini/maxi, kit name or protocol) Qiagen Maxi Prep protocol

Plasmid prep detail:

Maxi prep lysate and tip washing conditions Purification (Wash & elution)- Qia500 tips-

Strain used to produce plasmid

TOP-10

E. coli ori? Y/N

Υ

Contains Mycobacterial ori? Y/N

Final concentration

Dried, 20 µg/tube

Number of batches

3 x 20 µg

Buffer

TE Buffer

Method used for quantifying

A260

QC gel

Restriction enzymes used in QC analysis

Ndel, HindIII & Pstl

Expected size of restriction fragments

Vector

5.369kb pET28b Ndel + HindIII

Insert

0.920kb Ag85B Ndel + HindIII

Other

6.289kb Linearized plasmid- Ndel, HindIII, Pstl

Gel results:

All predicted bands visible pp. 131

Gel description (file number, % agarose, buffer)

ED Ag85B 2011.04.12.jpg, 1.5% 1 X TAE

Sequence file:

Ag85B sequence 02.01.2011.ppt

Plasmid Map: Attached

Generated by

Supervisor

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