SUPPORTING INFECTIOUS DISEASE RESEARCH

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: 61002002

Manufacturing Date: 12MAY2011

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for the bulk lot pMRLB76 02.03.2011 is attached. This lot was received as dry pellets from CSU and rehydrated in TE buffer (10 mM Tris-HCI, 1 mM EDTA buffer, pH 8) at ATCC[®]. Approximately 50 μ L of a 0.02 μ g/ μ L solution was dispensed per vial yielding a final amount of 1 μ g per vial. A plasmid map is attached.

ATCC[®], on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor 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[®] \leq knowledge.

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Recombinant Plasmid Quality Control Record

Plasmid designation Lot Number	pMRLB76 (pET28-Ag8 pMRLB76 02.03.2011	85B ML2028	<u>.)</u>		
Notebook/Pgs Notebook detail	JNKBook#1/ pp.123-126, 130 Plasmid Prep pp.123-126 A260 and stock dilution pp.126 QC gel pp. 130				
Media used Culture size Growth conditions:	<u>LB Broth 50 μg/mL Kar</u> <u>1 x 1L</u> Temp <u>37°C</u> Time	<u>namycin</u> <u>16 hr.</u>	Shaker speed	<u>130 rpm</u>	
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 <u>TOP-10</u>					
<i>E. coli</i> ori? Y/N	Y				
Contains Mycobacterial ori? Y/N N					
Final concentration	<u>Dried, 20 µg/tube</u>				
Number of batches Buffer	<u>4 x 20 μg</u> <u>TE Buffer</u>				
Method used for quantifying <u>A260</u>					
QC gel					
Restriction enzymes used in QC analysis Ndel, HindIII & Pstl					

Expected size of rest	riction 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 2011.02.23.jpg,1.5% 1 X TAESequence file:Ag85B sequence 02.01.2011.ppt

Plasmid Map: Attached

Generated by	Jade Funk Date 5/12/11	
Supervisor	Jahn Marcer Date 5/12/11	

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