

Certificate of Analysis for NR-36492

Mycobacterium tuberculosis, Strain East African Indian 91_0079, Gamma-Irradiated Whole Cells

Catalog No. NR-36492

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

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain East African Indian 91_0079 was grown to late-log phase in glycerol-alanine-salts medium and inactivated by exposure to 2.4 mRads of ionizing gamma irradiation using a ¹³⁷Cs source. Confirmation of inactivation was performed by Alamar Blue assay. A dose of 2.4 mRads of gamma irradiation kills *M. tuberculosis* to a 10²⁰ degree of certainty while maintaining 93% to 95% of the biological activity of the enzymes. The bacilli are harvested by filtration and washed with PBS pH 7.4.

Lot: 61977668 Manufacturing Date: 21AUG2013

Production and QC testing were performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for bulk lot 13.EAI.7.30.8.WCg is attached. This lot was aliquoted at Colorado State University and each vial contains approximately 10 g of *Mycobacterium tuberculosis*, strain East African Indian 91 0079 gamma-irradiated whole cells.

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®'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.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

WORK SHEET FOR γ-IRRADIATED CELLS

General Information Lot Number: 13.EAI.7.30.8.WCg Species: M. tuberculosis Strain: East African Indian 91 0079 **Growth Conditions:** Medium: GAS # of Fernbachs: 16 # of Roller bottles: 0 Inoculation Date: 7.30.13 Harvest Date: 8.13.13 SOP # used for Harvest: PP003.3 1/11/13 version Wet Weight: 83.2g Date irradiated: 8.21.13 By: IC Alamar Blue QC Information

Date Checked: 8.30.13 Date Started: 8.28.13

Positive Control: Positive X Negative

OD = .1 change: 0.0392

Sample: Positive Negative X

OD=.1 change: 0.0011

Aliquot Information

(Research Associate)

1 x 83.2g in 230ml falcon tube

(Laboratory Supervisor)

	0.76 0.75	0.73 0.72	0.74 0.73	0.75 0.74			100 OD:0001	0.01 0.00
	0.75	0.74	0.75	0.93		3lank	1 OD .001	0.00
48 hr 570/600						48hr Minus Blank	10. do	
48 hr 5	0.81	0.81	0.92	2.59		48	0D.1	90.0
0.00	8-30-13.							
	1.01	1.02	0.98	0.92			OD.0001	0.08
	96.0	0.97	0.94	06:0		24hr Minus Blank		0.03
009,	96.0	0.95	0.93	0.92			100. do 10. do	0.03
24hr 570/600	96'0	0.95	0.95	1.12			0D.1	0.03
	8-29-13.							
	1.06	1.06	1.01	0.94		Initial Minus Blank	OD.0001	60.0
	1.08	1.07	1.00	0.94				0.11
009/02	1.03	1.01	0.98	0.93			100. do 10. do	90'0
Initial 0 hr 570/600	1.03	1.02	66.0	0.93		Initial	0D.1	90'0
	8-28-13.	13.EAI.7.30.8.WCg						
		7.30						

	24h	24hr Minus Blank	¥			48h	48hr Minus Blank	¥	
	0D.1	10. do	0D .001	OD.0001		00.1	10. do	0D.001	OD:0001
	0.03	0.03	0.03	0.08		90:0	0.00	10.0	00.00
Sample	0.01	0.02	0.04	0.08	Sample	90.0	-0.01	-0.01	-0.03
	0.02	0.00	0.00	0.04		0.17	0.00	-0.01	-0.02
Pos. Cont.	0.18	-0.02	0.04	-0.01	Pos. Cont.	1.84	0.18	0.01	-0.01
	57	24hr Average				48	48hr Average		
	0D.1	0D .01	100. do	OD:0001	12 76	0D.1	10. do	0D .001	OD.0001
Sample	0.02	10.0	0.02	0.07	Sample	0.10	00.00	-0.01	-0.02
Pos Chri	0.18	-0 02	-0.04	-0.01	Pos Ctrl	184	0.18	100	-0.01

0.04

0.03

0.01

0.02

Pos. Cont.

| Initial Average | OD .01 | OD .01 | OD .001 | OD .001 | OD .001 | OD .008 | OD .008 | OD .008 | OD .002 | OD .002

Sample Pos. Ctrl.

 Sample .1
 Sample .01
 Pos .1
 Pos .01

 0
 0.05
 0.04
 -0.04
 -0.04

 24
 0.02
 0.01
 0.09
 -0.02

 48
 0.10
 0.00
 1.84
 0.18

	·· • · · Sample .1 · • • · Sample .0.1 - • • · Sample .0.1 - • • · · · · · · · · · · · · · · · · ·	Linear (Sample .1) Linear (Sample .01) ——Linear (Pos .1)	Linear (Pos. 01)	y = 0.0045x - 0.056 y = 0.0011x + 0.0287 y = 0.0011x + 0.0287	99 O
570/600 vs Time				11111111111111111111111111111111111111	30 40 50 Hours after setup
	2.00		SystayA Systamonon-minorca Systamon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamonon-minorca Systamo		07