

Certificate of Analysis for NR-48696

Mycobacterium tuberculosis, Strain H37Rv, Gamma-Irradiated Whole Cells Kit Containing Hypoxic and Normoxic Cultures

Catalog No. NR-48696

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

Product Description: NR-48696 is a kit comprised of gamma-irradiated cells from two preparations of *Mycobacterium tuberculosis* (*M. tuberculosis*), strain H37Rv.

Lot: 70024127

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Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for bulk lots 18.Rv.2.11.31.4.WCg.Hypoxic and 18.Rv.2.11.31.4.WCg.Normoxic is attached. The kit was assembled by BEI Resources on 15APR2019.

KIT COMPONENT	BEI RESOURCES PRODUCT AND LOT NUMBERS	CSU LOT NUMBER	MANUFACTURING DATE
M. tuberculosis, Strain H37Rv, Irradiated Cells (hypoxic culture)	NR-36493 lot 70017944	18.Rv.2.11.31.4.WCg.Hypoxic ¹	22DEC2018
M. tuberculosis, Strain H37Rv, Irradiated Cells (normoxic culture)	NR-36494 lot 70017945	18.Rv.2.11.31.4.WCg.Normoxic ²	19DEC2018

¹M. tuberculosis, strain H37Rv hypoxic culture was grown to early log phase in glycerol-alanine-salts medium for 4 days in a bioreactor. The cells were sparged with nitrogen gas to slowly deplete oxygen. The cells were grown an additional 10 days and harvested.

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BEI Resources E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

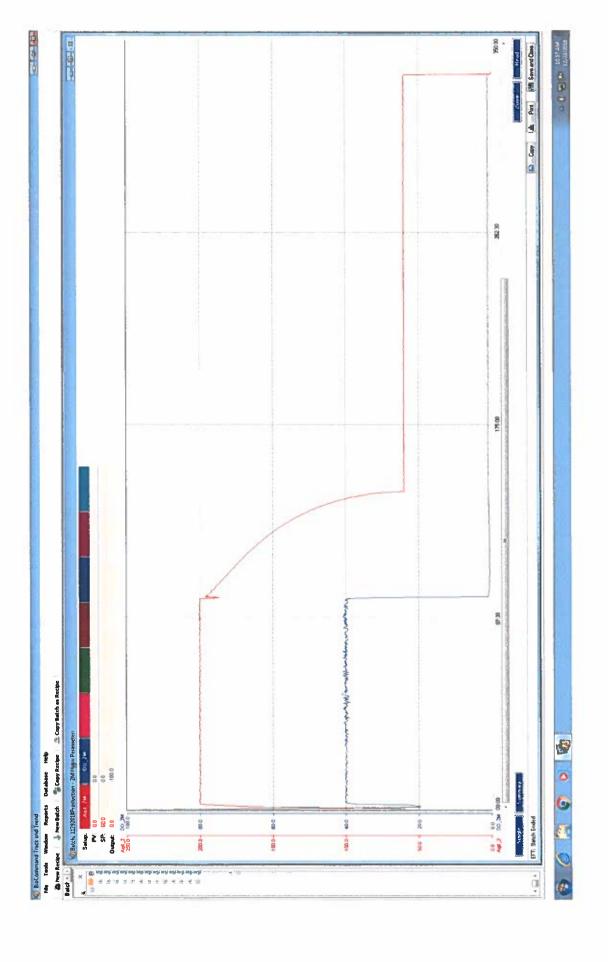
²M. tuberculosis, strain H37Rv normoxic culture was grown to mid log phase in glycerol-alanine-salts medium for 10 days in a bioreactor. The cells were sparged with air and harvested.

WORK SHEET FOR γ-IRRADIATED CELLS

General Information Lot Number: 18.Rv.2.11.31.4.WCg.Hypoxic Species: M. tuberculosis Strain: H37Rv **Growth Conditions:** Medium: GAS # of Fermentors: 1 (4L) Inoculation Date: 11/31/18 Harvest Date: 12/13/18 SOP # used for Harvest: PP040.1 Wet Weight: 28.1g Date irradiated: 12/22/18 By: AS **Alamar Blue QC Information** Date Started: 01/07/19 Date Checked: 01/09/19 Positive Control: Positive X Negative Sample: Positive Negative X **Aliquot Information** 1x 28.1g in 230ml conical tube. See graph for dissolved oxygen (DO) log. (Research Associate) (date) 1/14/19

(date)

(Laboratory Supervisor)



0/600	0.73	0.73	0.73	0.89
24hr 570/600	0.82	0.82	0.83	3.99
	1-8-19.			
	0.70	0.70	0.67	0.68
	69'0	0.70	69'0	69.0
009/	0.70	0.71	69'0	0.70
nitial 0 hr 570/600	0.78	0.79	0.79	0.75
itial (ı		
Initial	1-7-19.	8.Rv.2.11.31.4.Hypoxic.WC		

	Initial	Initial Minus Blank	Y	
	00.1	10. do	00 001	OD:0001
	0.08	-0.01	-0.02	-0.01
Sample	0.08	0.00	-0.01	-0.01
	0.08	-0.02	-0.02	-0.04
Pos. Cont.	0.04	-0.01	-0.02	-0.03

	Initial	Initial Minus Blank	k	
	1.00	10. GO	100.00	OD.0001
	0.08	-0.01	-0.02	-0.01
Sample	0.08	0.00	-0.01	-0.01
	0.08	-0.02	-0.02	0.04
Pos. Cont.	0.0	-0.01	-0.02	-0.03
	Init	Initial Average		
	00.1	OD 01	100.00	OD:0001
Sample	0.08	-0.01	-0.05	-0.02
Pos. Ctrl.	0.04	-0.01	-0.02	-0.03

Sample .1

		OD.0001	-0.01	-0.01	0.04	-0.03		OD:0001	-0.02	-0.03	
	k	100.00	-0.02	-0.01	-0.02	-0.02		100.00	-0.05	-0.02	
	nitial Minus Blank	10. GO	-0.01	0.00	-0.02	-0.01	Initial Average	OD 01	-0.01	-0.01	
١	nitial	-	0.08	0.08	0.08	0.04	Initi	1	0.08	0.04	

	24hr	24hr Minus Blank	k	
	1. 00	10. do	00 001	OD 0001
	0.10	0.01	00.00	00.00
Sample	0.10	0.01	00.00	0.00
	0.11	0.01	0.00	0.00
Pos. Cont.	3.27	0.17	0,01	0.00
	24	24hr Average		
	1, 00	10' QO	OD .001	OD.0001
Sample	0.11	0.01	00'0	0.00
Pos. Ctrl.	3.27	0.17	10.01	0.00

	48h	48hr Minus Blank	*	
	1.00	10. do	100.00	OD:0001
	0.13	0.01	0.00	-0.01
Sample	0.14	0.01	0.00	00'0
	0.13	0.01	0.00	00'0
Pos. Cont.	3.37	0.39	0.03	0.00
	48	48hr Average		
	1.00	10. 00	100. do	OD.0001
Sample	0.13	0.01	0.00	00'0
Pos. Ctrl.	3,37	0,39	0.03	0.00

0.73

0.73

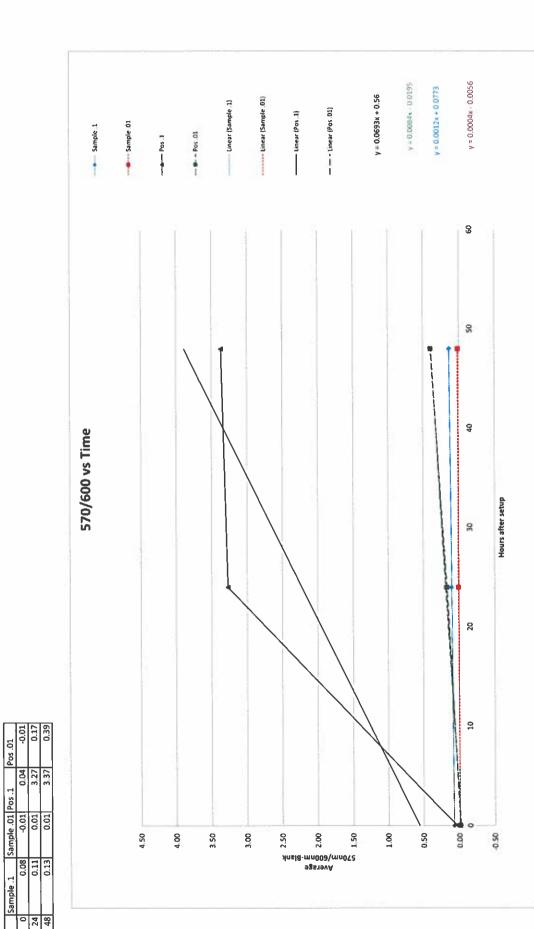
0.74 0.74 1.12

48 hr 570/600 0.86 0 0.87 0 4.10 1

1.9-19

0.72

0.72

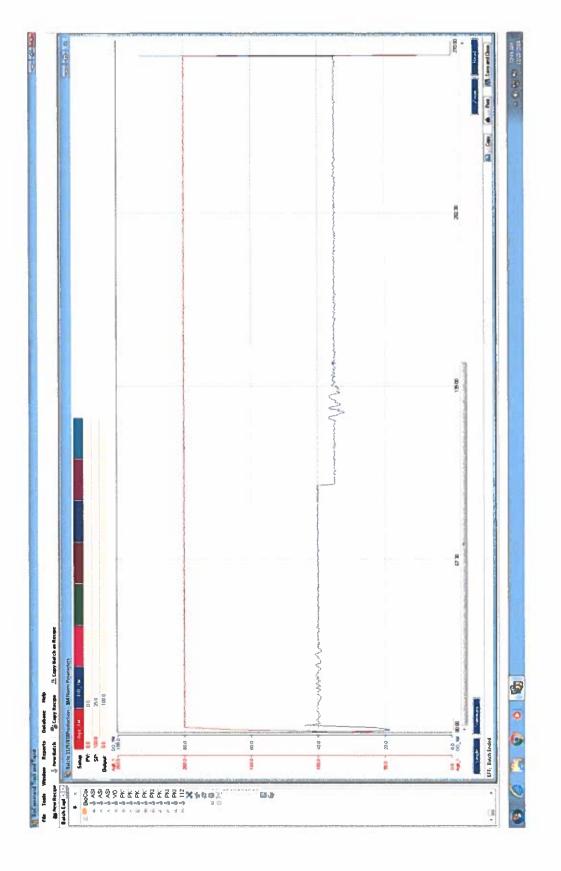


WORK SHEET FOR γ-IRRADIATED CELLS

General Information Lot Number: 18.Rv.2.11.31.4.WCg.Normoxic Species: M. tuberculosis Strain: H37Rv **Growth Conditions:** Medium: GAS # of Fermentors: 1 (4L) Inoculation Date: 11/31/18 Harvest Date: 12/10/18 SOP # used for Harvest; PP040.1 Wet Weight: 78.5g Date irradiated: 12/19/18 By: AS Alamar Blue QC Information Date Started: 01/07/19 Date Checked: 01/09/19 Positive Control: Positive X Negative Sample: Positive Negative X **Aliquot Information** 1x 78.5g in 230ml conical tube. See graph for dissolved oxygen (DO) log. Vessel harvested at day 11 (instead of day 10) due to schedule conflict. (Research Associate) (date) X C. Heliaffy 1/14/19

(date)

(Laboratory Supervisor)



			24hr	24hr 570/600					48 hr 570/600	900		
0	72	1-8-19.		0.77	0.72	0.72	0.72	1-9-19.	0.79	0.74	0.73	0.73
0.7	10		0	0.77	0.72	0.72	0.72		0.79	0.74	0.73	0.73
0.7	<u></u>		0	0.77	0.73	0.72	0.72		0.79	0.74	0.73	0.73
0.68	60		3.	3.99	68.0	0.73	0.71		4.10	1.12	0.76	0.73

		24hr 570/600	009/		
	1-8-19.	0.77	0.72	0.72	0.72
		0.77	0.72	0.72	0.72
		0.77	0.73	0.72	0.72
		3.99	0.89	0.73	0.71

raw 1-7-19. 18.Rv.2.11.31.4.Normoxic.W

ļ			L			Pos	l			San	Pos	
		OD.0001	0.01	0.01	0.02	-0.03			OD:0001	0.01	-0.03	
	ı V	100.00	0.01	-0.01	0.03	-0.02	1		100. OO	0.01	-0.02	
	Initial Minus Blank	10. do	10.0	0.01	0.02	0.01		Initial Average	0D :01	0.01	-0.01	
0.0	Initial	0D .1	0.05	0.04	0.07	0,04		Init	0D .1	0.06	0.04	

Sample Pos. Cont.
 Sample .1
 Sample .0.1
 Pos .1
 Pos .0.1

 0
 0.06
 0.01
 0.04
 -0.01

 24
 0.05
 0.01
 3.27
 0.17

 48
 0.06
 0.01
 3.37
 0.39

Sample Pos. Ctrl.

	4141	ETH WILLIAM CHOICE		
	00.1	10. OO	OD .001	OD:0001
	0.05	0.01	0.00	0.00
Sample	0.05	0.01	0.00	0.00
	0.05	0.01		0.00
Pos. Cont.	3.27	0.17	0.01	0.00
	24	24hr Average		
	1,00	10. 00	00 .001	OD:0001
Sample	0.05	0.01	0.00	00:00
Pos. Ctrl.	3.27	0.17	0.01	0.00

	48h	48hr Minus Blank	ık	
	1. 00	10. GO	100.00	OD:0001
	0.05	0.00	0.00	00.00
Sample	90.0	0.01	0.00	00.00
	90.06	0.01	0.00	0.00
Pos. Cont.	3.37	0.39	0.03	0.00
	48	48hr Average		
	1.00	10.00	00 .001	OD:0001
Sample	0.06	0.01	0.00	0.00
Pos. Ctrl.	3.37	0.39	0.03	00:00

Sample 1	Sample 01	- Marian	- Pos.01	Linear (Sample -1)		Linear (Pos. 1)		y = 0.0693x + 0.56	v = 0.0084x - 0.0195	y 9E.05x + 0.0537	y = -0.0002x + 0.013	
											99	
		,		1						•	95	7 (1)
vs Time		the early								100	040	
570/600 vs Time										1	30	
				\	\						20	
								1			10	
	4.50	4.00	3.50	80			909/mnore	1.00		950	00:00	0.50