

***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: 70052466

Manufacturing Date: 14FEB2022

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for bulk lots 22.Rv.2.1.14.8.WCg.Hypoxic and 22.Rv.2.1.14.8.WCg.Normoxic is attached. The kit was assembled by BEI Resources on 26APR2022.

KIT COMPONENT	BEI RESOURCES PRODUCT AND LOT NUMBERS	CSU LOT NUMBER	MANUFACTURING DATE
<i>M. tuberculosis</i> , Strain H37Rv, Irradiated Cells (hypoxic culture)	NR-36493 lot 70045516	22.Rv.2.1.14.8.WCg.Hypoxic ¹	14FEB2022
<i>M. tuberculosis</i> , Strain H37Rv, Irradiated Cells (normoxic culture)	NR-36494 lot 70045517	22.Rv.2.1.14.8.WCg.Normoxic ²	14FEB2022

¹*M. tuberculosis*, strain H37Rv hypoxic culture was grown to early log phase in glycerol-alanine-salts medium for 14 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.

²*M. 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.

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WORK SHEET FOR γ -IRRADIATED CELLS

General Information

Lot Number: 22.Rv.2.1.14.8.WCg.Hypoxic

Species: M. tuberculosis

Strain: H37Rv

Growth Conditions:

Medium: GAS

of Fermentors: 1 (3.5L)

Inoculation Date: 1.14.22 Harvest Date: 1.28.22

SOP # used for Harvest: PP0040.1

Wet Weight: 15.1g

Date irradiated: 2.14.22 By: JB

Alamar Blue QC Information

Date Started: 2.28.22 Date Checked: 3.2.22

Positive Control: Positive X Negative

Sample: Positive Negative X


Aliquot Information

1 x 15.1g in 230ml falcon tube

See graph for dissolved oxygen (DO) log.

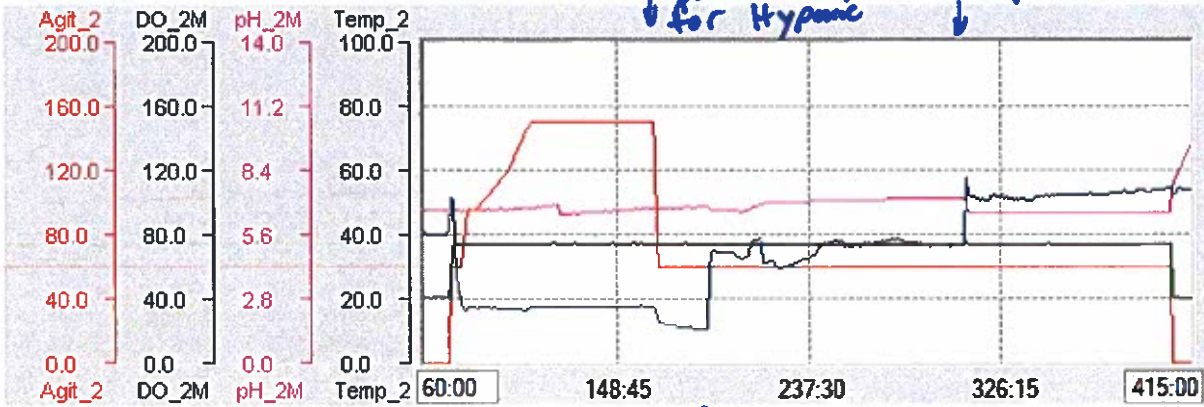


(Research Associate) 3-2-22
(date)



(Laboratory Supervisor) 3-3-22
(date)

Batch: BEI2022 - 2M Hypo Parameters

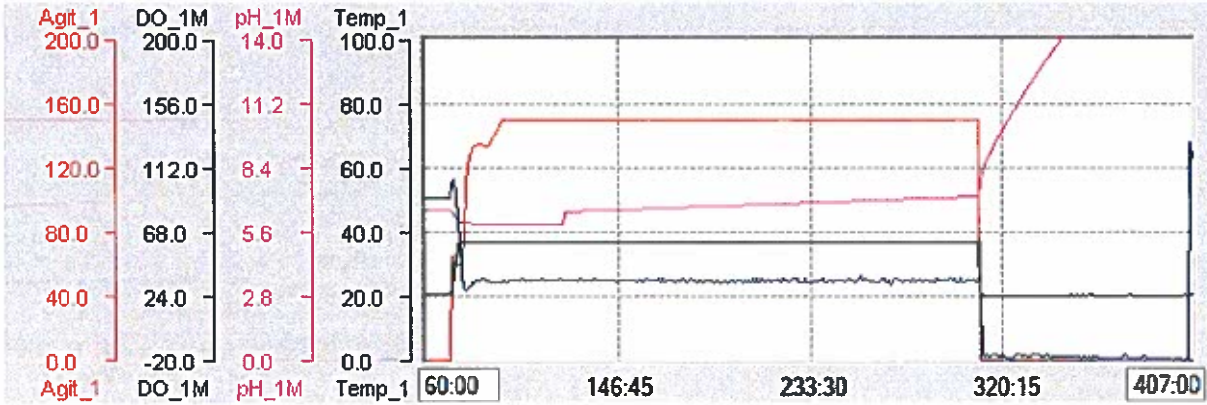


Start Cascade for Hypoxic
 DO probe replaced

- 2M DO probe nonfunctional
- Cascade still working
- Min cascade .2 to force N₂ and deplete O₂

Batch: BEI2022 - 1M Norm Parameters

1M DO Probe moved
↓ to 2M unit



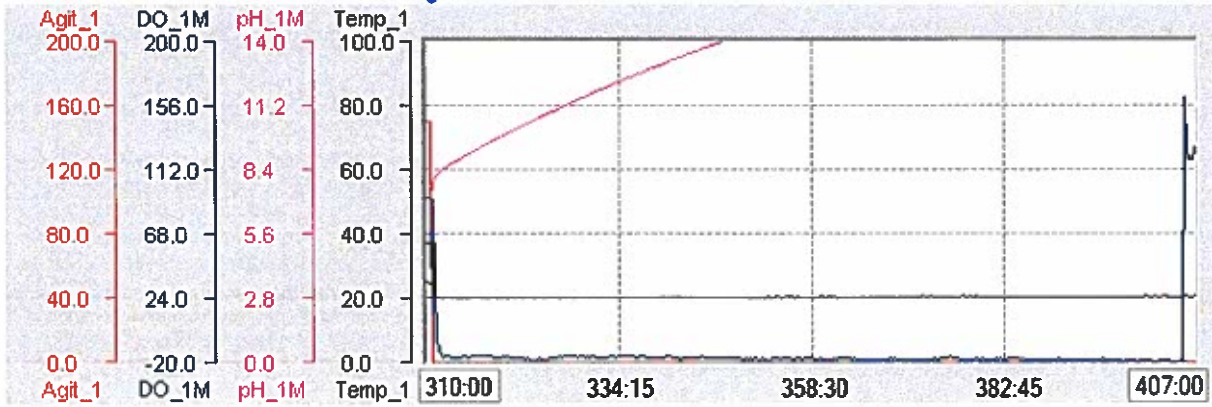
DO probe

Normoxic unit

Hypoxic unit

Batch: BEI2022 - 1M Norm Parameters

1M DO Probe moved to 2M unit



replacing nonfunctional 2M DO probe

raw	Initial 0 hr 570/600				
2-28-22.	0.77	0.72	0.73	0.70	0.70
22 Rv.2.1.14.8 WCGp_Hypocid	0.76	0.73	0.71	0.69	0.69
	0.78	0.74	0.71	0.69	0.69
	0.83	0.75	0.75	0.73	0.73

Initial Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.05	0.00	0.01	-0.01
	0.05	0.02	-0.01	-0.02
	0.07	0.02	-0.01	-0.03
Pos. Ctrl.	0.11	0.03	0.04	0.02

Initial Average				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.06	0.01	0.00	-0.02
Pos. Ctrl.	0.11	0.03	0.04	0.02

	Sample .1	Sample .01	Pos. .1	Pos. .01
0	0.06	0.01	0.11	0.03
24	0.04	0.01	2.53	0.19
48	0.05	0.01	2.57	0.56

24hr 570/600				
3-1-22.	0.79	0.76	0.75	0.75
	0.79	0.75	0.74	0.74
	0.79	0.75	0.74	0.74
	3.27	0.94	0.77	0.76

24hr Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.04	0.02	0.00	0.00
	0.04	0.00	-0.01	-0.01
	0.04	0.00	-0.01	-0.01
Pos. Ctrl.	2.53	0.19	0.02	0.01

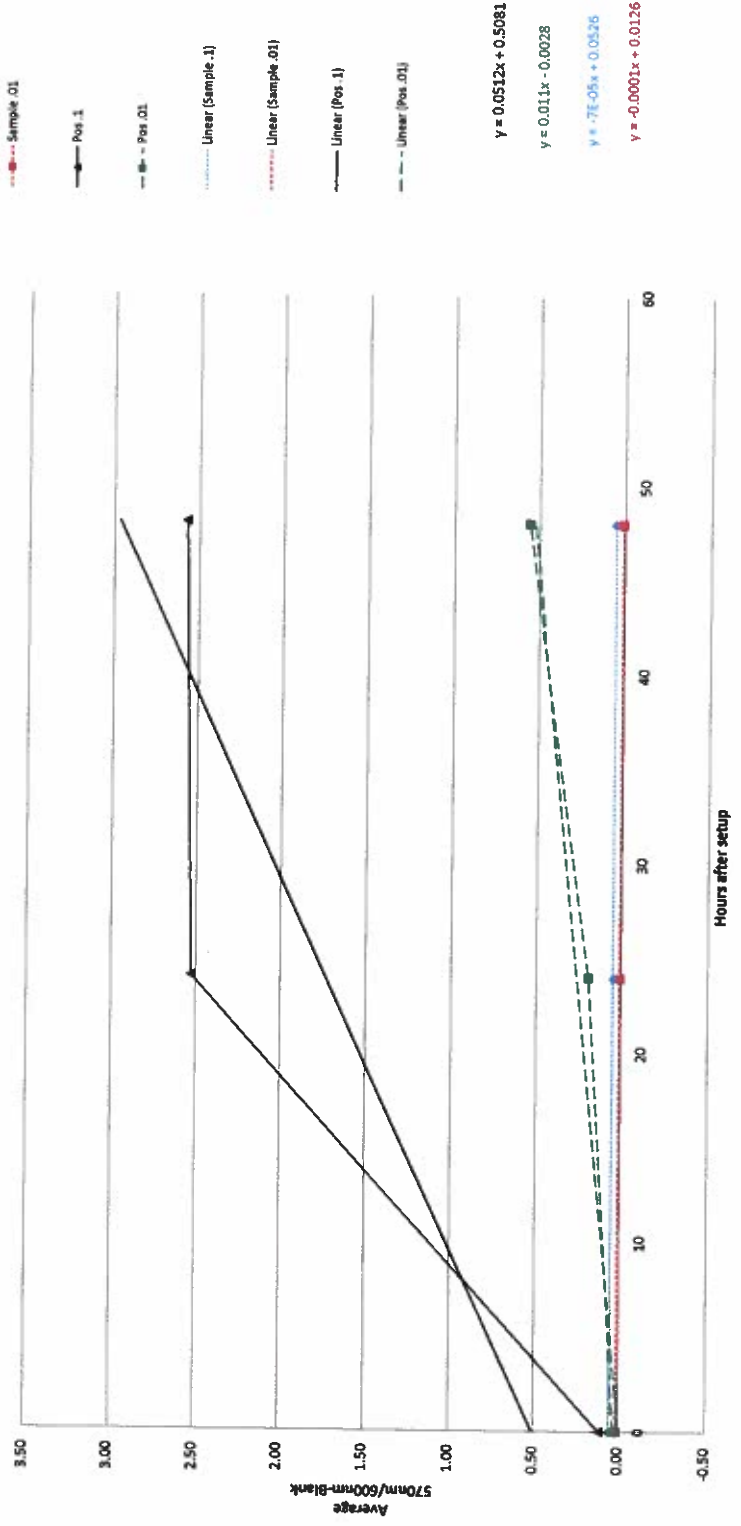
24hr Average				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.04	0.01	0.00	-0.01
Pos. Ctrl.	2.53	0.19	0.02	0.01

48 hr 570/600				
3-2-22.	0.83	0.79	0.77	0.77
	0.82	0.78	0.77	0.77
	0.83	0.78	0.77	0.77
	3.35	1.33	0.83	0.79

48hr Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.06	0.01	0.00	0.00
	0.05	0.00	-0.01	-0.01
	0.05	0.00	0.00	-0.01
Pos. Ctrl.	2.57	0.56	0.05	0.01

48hr Average				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.05	0.01	0.00	-0.01
Pos. Ctrl.	2.57	0.56	0.05	0.01

570/600 vs Time



WORK SHEET FOR γ -IRRADIATED CELLS

General Information

Lot Number: 22.Rv.2.1.14.8.WCg.Normoxic

Species: M. tuberculosis

Strain: H37Rv

Growth Conditions: Medium: GAS

of Fermentors: 1 (3.5L)

Inoculation Date: 1.14.22 Harvest Date: 1.24.22

SOP # used for Harvest: PP0040.1

Wet Weight: 23.4g

Date irradiated: 1.28.22 By: JB

Alamar Blue QC Information

Date Started: 2.28.22 Date Checked: 3.2.22


Positive Control: Positive X Negative


Sample: Positive Negative X

Aliquot Information

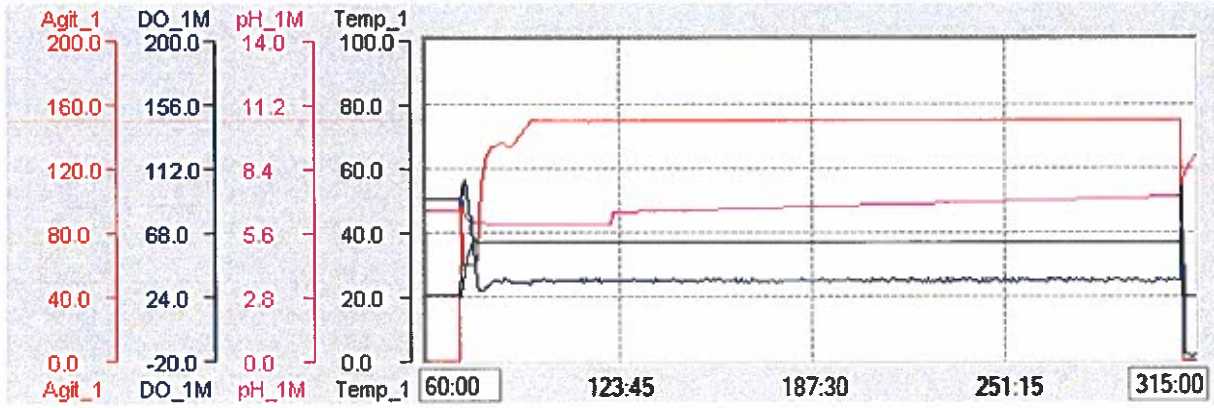
1 x 23.4g in 230ml falcon tube

See graph for dissolved oxygen (DO) log.

 3-2-22
(Research Associate) (date)

 3-3-22
(Laboratory Supervisor) (date)

Batch: BEI2022 - 1M Norm Parameters



RAW	Initial 0 hr 570/600				
	2-28-22	0.76	0.72	0.73	0.72
22.Rv.2.1.14.8.WCg.Normox		0.77	0.73	0.74	0.72
		0.77	0.72	0.74	0.72
		0.83	0.75	0.75	0.73

Initial Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.05	0.01	0.01	0.01
	0.06	0.02	0.03	0.00
	0.06	0.01	0.02	0.00
Pos. Cont.	0.11	0.03	0.04	0.02

Initial Average				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.05	0.01	0.02	0.00
Pos. Ctrl.	0.11	0.03	0.04	0.02

	Sample .1	Sample .01	Pos. .1	Pos .01
0	0.05	0.01	0.11	0.03
24	0.04	0.01	2.53	0.19
48	0.05	0.01	2.57	0.56

24hr 570/600					
	3-1-22	0.78	0.76	0.75	0.75
		0.79	0.76 <td>0.75 <td>0.75</td> </td>	0.75 <td>0.75</td>	0.75
		0.79	0.76 <td>0.75 <td>0.75</td> </td>	0.75 <td>0.75</td>	0.75
		3.27	0.94	0.77	0.76

24hr Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.04	0.01	0.00	0.01
	0.04	0.01	0.00	0.00
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48 hr 570/600					
	3-2-22	0.82	0.78	0.78	0.78
		0.83	0.78 <td>0.77 <td>0.78</td> </td>	0.77 <td>0.78</td>	0.78
		0.82	0.78 <td>0.78 <td>0.78</td> </td>	0.78 <td>0.78</td>	0.78
		3.35	1.33	0.83	0.79

48hr Minus Blank				
	OD .1	OD .01	OD .001	OD.0001
Sample	0.05	0.01	0.00	0.01
	0.05	0.01	0.00	0.00
	0.05	0.01	0.01	0.01
Pos. Cont.	2.57	0.56	0.05	0.01

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	OD .1	OD .01	OD .001	OD.0001
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570/600 vs Time

