

Certificate of Analysis for NR-31210

Mycobacterium bovis, Strain AF 2122/97 (ATCC[®] BAA-935[™]), Gamma-Irradiated Whole Cells

Catalog No. NR-31210

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

Product Description: *Mycobacterium bovis* (M. bovis), strain AF 2122/97 (ATCC[®] BAA-935TM), was grown to late-log phase in sodium pyruvate 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. bovis to a 10^{20} 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: 62554357 Manufacturing Date: 12JAN2015

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.BAA935.12.11.6.WCg 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®'s knowledge.

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WORK SHEET FOR γ-IRRADIATED CELLS

General Information Lot Number: 14.BAA935.12.11.6.WCg Species: M. bovis Strain: BAA935 Medium: SPAS **Growth Conditions:** # of Fernbachs: 16 # of Roller bottles: 0 Inoculation Date: 12.11.14 Harvest Date: __1.1.15 SOP # used for Harvest: PP003.4 6.16.14 version Wet Weight: 27.2g Date irradiated: 1.12.15 By: RS Alamar Blue QC Information Date Checked: 2.20.15 Date Started: 2.18.15 Positive Control: Positive X Negative Sample: Positive_____ Negative__X__ **Aliquot Information** 27.2g in 230ml falcon tube

(Research Associate)

(Laboratory Supervisor)

| 48 hr 570/600 | 77 0.75 | 98 0.77 0.75 | 96 0.76 0.75 | 1.98 0.85 | | 48hr Minus Blank | 100. do 10. do | 3 0.02 0.00 | 94 0.02 0.00 | 11 0.02 0.00 | 01.0 1.23 0.10 | 48hr Average | 100. do 10. do | 3 0.02 0.00 |
|----------------------|----------|----------------------|--------------|-----------|--|---------------------|-----------------|-------------|--------------|--------------|----------------|-----------------|------------------|-------------|
| 48 hr | 0.97 | 0.98 | 0.96 | 7.74 | | 48 | 0D.1 | 0.23 | 0.24 | 0.21 | 7.00 | | 0D.1 | 0.23 |
| | 2-20-15. | | | | | | | | Sample | | Pos. Cont. | | | Sample |
| | | | | | | | | | | | | | | |
| | 0.74 | 0.74 | 0.74 | 0.74 | | | OD.0001 | 0.00 | 0.00 | 0.00 | 0.00 | | OD.0001 | 00.0 |
| | 0.74 | 0.74 | 0.74 | 0.82 | | 24hr Minus Blank | - | 0.00 | 0.00 | 0.00 | 0.08 | | 0D .001 | 0.00 |
| 009/ | 0.75 | 0.75 | 0.75 | 1.64 | | | 0D .00 DD .001 | 0.02 | 0.02 | 0.05 | 0.90 | 24hr Average | 10. do | 0.02 |
| 24hr 570/600 | 06.0 | 06:0 | 06.0 | 8.06 | | | . OD .1 | 0.16 | 0.17 | 0.16 | 7.32 | 24} | 0D .1 | 0.16 |
| | 2-19-15. | | | | | | | | Sample | | Pos. Cont. | | | Sample |
| | 5 0.74 | 1 0.74 | 3 0.72 | 0.72 | | | OD .001 OD.0001 | 0.01 | 0.01 | -0.01 | -0.01 | | OD.0001 | 00:00 |
| Initial 0 hr 570/600 | 0.75 | 0.74 | 0.73 | 0.75 | | Initial Minus Blank | OD .001 | 0.02 | | 0.00 | 0.02 | | OD .001 | 0.01 |
| | 0.76 | 0.73 | 0.73 | 0.73 | | | OD .01 | 0.03 | 0.00 | 0.00 | 0.00 | Initial Average | 0D .01 | 0.01 |
| | 0.74 | 0.75 | 0.75 | 0.82 | | Initia | 0D .1 | 0.01 | 0.02 | 0.03 | 0.09 | Init | 0D .1 | 0.02 |
| | 15. | 4.BAA935.12.11.6.WCg | | | | | | | Sample | | Pos. Cont. | | | Sample |
| | 2-18-15. | 12.11 | | | | | | _ | _ | | 4 | | Ш | S |

| | 0.75 | 0.75 | 7.74 1.98 0.85 0.76 | 48hr Minus Blank | OD .001 OD.0 | 0.02 | 0.00 | 1.23 0.10 | je | 3 00 | | | Sample .1 | Sample .01 | Pos .1 | — ■ – Pos .01 | | Linear (Sample .01) | —————————————————————————————————————— | —————————————————————————————————————— | | y = 0.1439x + 1.3498 | y = 0.0256x + 0.0976 | y = 0.0043x + 0.0326 | y = 0.0002x + 0.0095 |
|----------------------|----------|------|---------------------|---------------------|--------------|--------|-------|------------|-----------------|--------|------------|----------------------------------|-----------------|--------------------|--------|---------------|------|---|--|--|------|----------------------|----------------------|----------------------|-------------------------|
| ╟ | 2-20-15. | | | | | Sample | | Pos. Cont. | | + | Pos. Ctrl. | | | | | | | | | | | | | | 09 |
| | 414 | | | | | 0.0 | 0 | 0 | | 10 | | | | | | | 1 | | | | | | 1 7 | • | 20 |
| | 0.74 | | 0.74 | | 0D.0 | | 0.00 | | 1000 | 0.00 | | | | | | | | | | | | | 11 | | |
| | 0.74 | 0.74 | 0.82 | _ | OD. | 0.00 | 0.00 | 0.08 | 100 | 00.00 | 0.08 | | ae E | | | | | | | | | | | | 40 |
| 009 | 0.75 | 0.75 | 1.64 | 24hr Minus Blank | 0D .01 | 0.02 | 0.05 | 0.90 | a l | 0.02 | 0.90 | e | 0 vs Tii | | | | | | | | | | 11 | | |
| 24hr 570/600 | 06.0 | 06.0 | 8.06 | 24hr N | 0D .1 | 0.16 | 0.16 | 7.32 | 24h | 9 | 7.32 | | 570/600 vs Time | | | | | | | | | | | | 30 Hours after setup |
| | 7-19-15. | | | | | Sample | | Pos. Cont. | | Sample | Pos. Ctrl. | | | | | | | | | | | | 11 | | Hours a |
| | | | | | | | | | | | | | | | | | | | | | | | | 2 1 | 20 |
| | 0.74 | 0.72 | 0.72 | | OD.0001 | 0.01 | -0.01 | -0.01 | 1000 | 0.00 | -0.01 | | | | | | | | | | | | 1 | | |
| - | 0.75 | 0.73 | 0.75 | | | 0.02 | 0.00 | 0.02 | 001 | - | 0.02 | Pos.01 0.00 0.90 1.23 | | | | | | VINEAL DESIGNATION OF A PARTY OF | | | 1 | | | ==== | 10 |
| 00 | 0.73 | 0.73 | 0.73 | Tus Blank | - 20 | 0.03 | 0.00 | 0.00 | | - | 0.00 | 7.32 | | | | | | | | | | 1 | | | |
| Initial 0 hr 570/600 | 0.75 | 0.75 | 0.82 | Initial Minus Blank | | 0.01 | 0.03 | 0.09 | Initial Average | 12 | 0.09 | Sample .01 Pos .1 0.01 0.02 0.02 | | 9.00 | 8.00 | | 7.00 | 00.9 | 5.00 | | 3.00 | 2:00 | 1.00 | 0:00 | 0 |
| 드 | 5 | | H | | | ple | | j. | | 1 | | .1 Si 0.02 0.16 0.23 | | | | | | | ısla-mr | s19vA 1008\mn072 | | | | | |