

***Mycobacterium tuberculosis*, Strain Erdman Barcode Library**

**Catalog No. NR-50781**

**Lot No. 70007489**

**Manufacturing Date: 26SEP2017**

**For research use only. Not for human use.**

**Contributor:**

Sarah Fortune, Professor, M.D., Harvard T.H. Chan School of Public Health, Harvard University, Cambridge, Massachusetts, USA and Dirk Schnappinger, Ph.D., Department of Microbiology and Immunology, Weill Cornell Medical College, New York, New York, USA

**Manufacturer:**

Sarah Fortune, Professor, M.D., Harvard T.H. Chan School of Public Health, Harvard University, Cambridge, Massachusetts, USA

**Product Description:**

NR-50781 is a digitally barcoded *Mycobacterium tuberculosis* (*M. tuberculosis*), strain Erdman library where each bacterium in the library carries a unique, randomized sequence tag that can be used to quantitatively track the fate of each infecting bacterium. The “barcode” consists of a random 7-mer and adjacent 75-mer library identifier tag inserted at the bacterial attB L5 insertion site of a chromosomally integrating plasmid with pJEB402 backbone.<sup>1,2</sup> The plasmid was a standard integrating mycobacterial vector containing an *Escherichia coli* origin of replication, the attP element for insertion into the L5 site and is maintained by a kanamycin resistance cassette.<sup>1</sup> The strain remains susceptible to all first line antimycobacterial agents once the barcode is stably inserted into the bacterial chromosome. To quantitate barcodes via sequencing, the barcode is amplified from isolated genomic DNA in two rounds of nested PCR steps.<sup>2,3</sup>

The virulence characteristics of the barcoded *M. tuberculosis* have been assessed by quantitative plating and assessments of pathology in over two dozen nonhuman primates where the barcoded strain has similar virulence characteristics to the parental strain of Erdman.<sup>2</sup> The complete genome of *M. tuberculosis*, strain Erdman was deposited in the DNA Database of Japan under accession no. AP012340.<sup>1</sup>

**Material Provided:**

Each vial contains approximately 0.7 mL (1.45 × 10<sup>8</sup> cfu) of *M. tuberculosis*, strain Erdman barcode library in Middlebrook 7H9 medium with additives (10% oleic acid-dextrose-catalase; 0.2% glycerol; 0.05% Tween® 80 and 20 µg/mL kanamycin) and cryopreservative (10% glycerol, 0.85% NaCl and 0.05% tyloxapol). The stock can be used directly for infection or can be recovered in broth culture prior to infection, depending on the lab’s infection protocol.

**Growth Conditions:**

If the bacteria are to be recovered, the entire vial should be inoculated into Middlebrook 7H9 medium (Appendix I).

**Note:** Do not repeatedly culture the same aliquot. This can skew qtag/barcode (qbid) distribution. Expansion of the culture is the same for all but the protocol for infection will vary depending on the route of infection. Users should follow their lab protocol once the culture is expanded.

**Media:**

Middlebrook 7H9 medium (with the addition of 10% oleic acid-dextrose-catalase; 0.2% glycerol; 0.05% Tween-80). The barcoded Erdman library is kanamycin-resistant and should be grown in the presence of 20 µg/mL kanamycin.

**Incubation:**

Temperature: 37°C

Atmosphere: Aerobic with 5% CO<sub>2</sub>

**Preparation:**

1. Keep vial frozen until ready for use, then thaw aliquot at room temperature.
2. Transfer the entire thawed aliquot into 12 mL of media.
3. Incubate at 37°C until OD<sub>600</sub> reaches 0.8.
4. Sonicate 3 times for 5 seconds each at 40% power to disrupt the clumps and get a single cell suspension.
5. Follow lab protocol for infection.

**Packaging/Storage:**

NR-50781 was packaged aseptically in cryovials. The product is provided frozen on dry ice and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Mycobacterium tuberculosis*, Strain Erdman Barcode Library, NR-50781.”

**Biosafety Level: 3**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

**Disclaimers:**

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at [www.beiresources.org](http://www.beiresources.org).

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any

warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

**Use Restrictions:**

**This material is distributed for internal research, non-commercial purposes only.** This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale. This material may be subject to third party patent rights.

**References:**

1. Fortune, S., Personal Communication.
2. Blumenthal, A., et al. "Simultaneous Analysis of Multiple *Mycobacterium tuberculosis* Knockdown Mutants *In Vitro* and *In Vivo*." *PLoS One* 5 (2010): e15667. PubMed: 21203517.
3. Martin, C. J., et al. "Digitally Barcoding *Mycobacterium tuberculosis* Reveals *In Vivo* Infection Dynamics in the Macaque Model of Tuberculosis. *mBio* 8 (2017): e00312-17. PubMed: 28487426.

ATCC® is a trademark of the American Type Culture Collection.



**APPENDIX I: MIDDLEBROOK 7H9 MEDIUM**

1. Prepare the 7H9 salts stock following the recipe below:

7H9 Salts Stock	
Ammonium Sulfate	0.5 g
Monopotassium Phosphate	2.5 g
Disodium Phosphate	1.0 g
Sodium Citrate	0.1 g
Magnesium Sulfate	0.05 g
Calcium Chloride	0.0005 g
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g
Ferric Ammonium Citrate	0.04 g
L-Glutamic Acid	0.5 g
Pyridoxine	0.001 g
Biotin	0.0005 g

2. Determine the final volume of Middlebrook M7H9 medium to prepare (1 L, 750 mL or 500 mL). Combine the 7H9 salts with glycerol and deionized water following the recipe below for the desired final volume of Middlebrook M7H9 medium, and mix well.

	To make:		
	1L	750 mL	500 mL
7H9 Salts Stock	4.7 g	3.525 g	2.35 g
50% Glycerol	4 mL	3 mL	2 mL
Distilled, Deionized Water	900 mL	675 mL	450 mL

3. To the volume of Middlebrook 7H9 medium made in step 2, transfer the components to the biocontainment hood and add the amounts of 20% Tween<sup>®</sup> 80 and 10× ADC or OADC to the corresponding volume of Middlebrook 7H9 medium:

Middlebrook M7H9 (made in step 2)	1L	750 mL	500 mL
20% Tween <sup>®</sup> 80	2.5 g	1.875 g	1.25 g
ADC <sup>1</sup> or OADC <sup>2</sup>	100 mL	75 mL	50 mL

<sup>1</sup>Middlebrook ADC (albumin, dextrose, catalase) Enrichment is commercially available (Sigma, M0678; BD, 212352)

<sup>2</sup>Middlebrook OADC (oleic, albumin, dextrose, catalase) Enrichment is commercially available (Sigma, M0553; BD, 212351)

4. Adjust the pH to 6.6 ± 0.2 at 25°C and filter sterilize using a 0.22 µm filter.

**Notes:**

1. Alternatively, the Middlebrook 7H9 medium prepared in step 2 can be autoclaved. The OAD or OADC components may be added, in the biocontainment hood, once the medium is cool to the touch.
2. Middlebrook 7H9 medium is a liquid growth medium specially used for culture of *Mycobacterium*. Middlebrook 7H9 medium supports the growth of mycobacterial species when supplemented with nutrients such as glycerol, oleic acid, albumin and dextrose.