

## **Certificate of Analysis for NR-18985**

## Mycobacterium tuberculosis, Strain HN353

## Catalog No. NR-18985

Product Description: Mycobacterium tuberculosis (M. tuberculosis), isolated in 1995 from the pulmonary tissue of a patient with tuberculosis in Texas, USA. Strain HN353 was deposited as a non-drug resistant strain.

Lot<sup>1</sup>: 61538745 Manufacturing Date: 03APR2013

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>2</sup>		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology <sup>3</sup>	Report results	Irregular, flat, undulate, opaque, rough and cream (Figure 1)
Growth on Brain Heart Infusion agar	Report results	Growth
Growth on MacConkey agar (without crystal violet)	No growth	No growth
Growth rate	≥ 7 days	≥ 7 days
Growth at 26°C	Negative	Negative
Growth at 37°C	Positive	Positive
Growth at 45°C	Negative	Negative
Growth at 55°C	Negative	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		, , , ,
Niacin production <sup>4</sup>	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Urease <sup>5</sup>	Report results	Negative
Aryl sulfate (3 days)	Negative	Negative
Aryl sulfate (14 days)	Positive	Negative <sup>6</sup>
Catalase	Positive	Positive
Iron uptake	Negative	Negative
Tween 80 hydrolysis	Report results	Negative
Growth in the presence of 5% sodium chloride	Negative	Negative
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Positive	Positive
Genotypic Analysis		7
Sequencing of HSP65 gene (~ 420 base pairs)	Consistent with M. tuberculosis	Consistent with <i>M. tuberculosis</i>
Purity (post-freeze) <sup>8</sup>	Consistent with M. tuberculosis	Consistent with M. tuberculosis
Viability (post-freeze) <sup>3</sup>	Growth	Growth

NR-18985 was produced by inoculation of the deposited material into Middlebrook 7H10 agar with OADC enrichment slants in an aerobic atmosphere with 5% CO2 for 26 days at 37°C. The material from the initial growth was passaged once in Middlebrook 7H9 broth with ADC enrichment for 29 days under propagation conditions.

**BEI Resources** 

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

Fax: 703-365-2898

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<sup>&</sup>lt;sup>2</sup>Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria, Biochemical Testing" Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, Available from: http://www.intechopen.com/books/biochemicaltesting/biochemical-isolation-and-identification-of-mycobacteria and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus Mycobacterium and for Description of New Slowly Growing Mycobacterium Species." Int. J. Syst. Bacteriol. 42 (1992): 315-323. PubMed:

<sup>&</sup>lt;sup>3</sup>14 days at 37°C in an aerobic atmosphere with 5% CO₂ on Middlebrook 7H10 agar with OADC enrichment

<sup>&</sup>lt;sup>4</sup>All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

<sup>&</sup>lt;sup>5</sup>>85% of *M. tuberculosis* strains are positive for urease activity.



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<sup>6</sup>Most slow-growing *M. tuberculosis* test positive for aryl sulfate after 14 days, but very slow growers may still show a negative result.

<sup>7</sup>Also consistent with M. africanum, M. bovis, M. canetti and M. microti

Figure 1



Date: 24 SEP 2014

Signature: (

Title: Technical Manager, BEI Authentication or designee

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

<sup>&</sup>lt;sup>6</sup>Purity was checked at 7,14, 21 and 28 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Middlebrook 7H10 agar with OADC enrichment.