

## **Certificate of Analysis for NR-50332**

## Cryptococcus neoformans, Strain NIH306

## Catalog No. NR-50332

**Product Description:** Cryptococcus neoformans (C. neoformans), strain NIH306 was isolated in San Gabriel, California in 1969 from human cerebrospinal fluid. Note: The label incorrectly refers to NR-50332 as strain NIH-306 14508722. The correct strain name for NR-50332 is NIH306.

Lot1: 64362156 Manufacturing Date: 08AUG2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology <sup>2</sup>	Report results	Circular yeast form cells, budding (Figure 1A)
Colony morphologies <sup>2</sup>	Report results	Colony type 1: (capsular) Circular, convex, entire margin, shiny, smooth and white (Figure 1B) Colony type 2: (acapsular) Circular, convex, entire margin, butyrous and translucent (Figure 1B)
CGB agar characterization <sup>3</sup>		
C. neoformans, strain NIH306 (NR-50332)	Yellow (no color change)	Yellow (no color change)
Positive control ( <i>C. neoformans</i> ; ATCC <sup>®</sup> MYA-4564 <sup>™</sup> )	Yellow (no color change)	Yellow (no color change)
Negative control (C. gattii; ATCC® MYA-4560™)	Blue	Blue
Genotypic Analysis		
Sequencing of partial 18S ribosomal RNA (rRNA) gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 26S rRNA (~ 520 base pairs) Sequencing of 28S rRNA gene (~ 620 base pairs)	≥ 99% sequence identity to C. neoformans type strain (GenBank: EU240005.1) ≥ 99% sequence identity to C. neoformans type strain (GenBank: KU729166.1)	99.4% sequence identity to C. neoformans type strain (GenBank: EU240005.1) 99.5% sequence identity to C. neoformans type strain (GenBank: KU729166.1)
Confirmation of Serotype A (C. neoformans var. grubii)4	(Genbank, 10725100.1)	(Genbank: No723100.1)
28S ribosomal RNA gene, partial sequence; Intergenic spacer (IGS) 1, partial sequence (1310 base pairs)	≥ 97% sequence identity to C. neoformans var. grubii	98.5% sequence identity to C. neoformans var. grubii (GenBank: CP003821.1)
5S rRNA gene (partial sequence) and IGS 2 (partial sequence) (~ 1110 base pairs)	≥ 97% sequence identity to C. neoformans var. grubii	98% sequence identity to C. neoformans var. grubii (GenBank: EF211262.1)
Confirmation of Fluconazole Susceptibility <sup>5</sup>	Sensitive (MIC ≤ 8 μg/mL)	Sensitive (MIC = 6 μg/mL)
Puritv <sup>6</sup>		
Nutrient broth with 0.1% Yeast Extract at 25°C	No bacterial growth	No bacterial growth
Nutrient broth with 0.1% Yeast Extract at 25 C	No bacterial growth	No bacterial growth
NUMBER DIONI WINTO. 170 TEAST EXHAUTAL ALST	No bacterial growth	No pacienal glowin
Viability (post-freeze) <sup>2</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>NR-50332 was produced by inoculation of the deposited material onto Yeast Mold slants and grown 2 days at 25°C in an aerobic atmosphere. Cells were harvested from the slants with 20% glycerol to produce this lot.

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<sup>&</sup>lt;sup>2</sup>5 days at 25°C in an aerobic atmosphere on Yeast Mold medium

<sup>35</sup> days at 25°C in an aerobic atmosphere. CGB medium differentiates C. gattii from C. neoformans based on the ability of C. gattii isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source, resulting in a blue color. C. neoformans isolates will remain yellow. [McTaggart, L., et al. "Rapid Identification of Cryptococcus neoformans var. grubii, C. neoformans var. neoformans, and C. gattii by Use of Rapid Biochemical Tests, Differential Media, and DNA Sequencing." J. Clin. Microbiol. 2011 (49): 2522-2527. PubMed: 21593254.]

<sup>&</sup>lt;sup>4</sup>C. neoformans subspecies can be differentiated by IGS sequence analysis; > 4% divergence is expected between species [McTaggart, L., et al. "Rapid Identification of Cryptococcus neoformans var. grubii, C. neoformans var. neoformans, and C. gattii by Use of Rapid Biochemical Tests, Differential Media, and DNA Sequencing." <u>J. Clin. Microbiol.</u> 2011 (49): 2522-2527. PubMed: 21593254.] <sup>5</sup>For fluconazole (bioMérieux Etest<sup>®</sup> 510858) a MIC ≤ 8 µg/mL is sensitive and a MIC ≥ 64 µg/mL is resistant.

<sup>&</sup>lt;sup>6</sup>Clarity of broth was determined by visual inspection after 15 days in an aerobic atmosphere.



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Figure 1: Cellular and Colony Morphologies

Colony Type 1

Colony Type 2

**Date:** 04 APR 2017

Signature: / fall mon

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