b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50333

Cryptococcus neoformans, Strain NIH398

Catalog No. NR-50333

Product Description: *Cryptococcus neoformans (C. neoformans),* strain NIH398 was isolated in Baltimore, Maryland in 1970 from human cerebrospinal fluid. Note: The label incorrectly refers to NR-50333 as strain NIH-398 14508722. The correct strain name for NR-50333 is NIH398.

Lot[1]: 64362159 **Manufacturing Date: 03AUG2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology[2]	Report results	Circular yeast cells, budding (Figure 1A)
Colony morphology[2]	Report results	Circular, slightly raised, entire margin, rough, white (Figure 1B)
CGB agar characterization[3]		
C. neoformans, strain NIH398 (NR-50333)	Yellow (no color change)	Yellow (no color change)
Positive control (*C. neoformans;* ATCC® MYA-4564™)	Yellow (no color change)	Yellow (no color change)
Negative control (*C. gattii*; ATCC® MYA-4563™)	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)	≥ 99% sequence identity to *C. neoformans* type strain (GenBank: EU240005.1)	99.4% sequence identity to *C. neoformans* type strain (GenBank: EU240005.1)
Sequencing of 28S rRNA gene (~ 620 base pairs)	≥ 99% sequence identity to *C. neoformans* type strain (GenBank: KU729166.1)	99.5% sequence identity to *C. neoformans* type strain (GenBank: KU729166.1)
Confirmation of Serotype A (*C. neoformans* var. *grubii*)[4]		
28S ribosomal RNA gene, partial sequence; Intergenic spacer (IGS) 1, partial sequence (~ 1200 base pairs)	≥ 97% sequence identity to *C. neoformans* var. *grubii*	99.9% sequence identity to *C. neoformans* var. *grubii* (GenBank: CP003821.1)
5S rRNA gene (partial sequence) and IGS 2 (partial sequence) (~ 1120 base pairs)	≥ 97% sequence identity to *C. neoformans* var. *grubii*	100% sequence identity to *C. neoformans* var. *grubii* (GenBank: CP003821.1)
Confirmation of Fluconazole Susceptibility[5]	Sensitive (MIC ≤ 8 µg/mL)	Sensitive (MIC = 4 - 6 µg/mL)
Purity[6]		
Nutrient broth with 0.1% Yeast Extract at 25°C	No bacterial growth	No bacterial growth
Nutrient broth with 0.1% Yeast Extract at 37°C	No bacterial growth	No bacterial growth
Viability (post-freeze)[7]	Growth	Growth

[1]NR-50333 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.
[2]5 days at 25°C in an aerobic atmosphere on Yeast Mold medium
[3]5 days at 26°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.]
[4]*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." J. Clin. Microbiol. 2011 (49): 2522-2527. PubMed: 21593254.]
[5]For fluconazole (bioMérieux Etest® 510858) a MIC ≤ 8 µg/mL is sensitive and a MIC ≥ 64 µg/mL is resistant.
[6]Clarity of broth was determined by visual inspection after 15 days in an aerobic atmosphere.
[7]2 days at 25°C in an aerobic atmosphere on Yeast Mold medium

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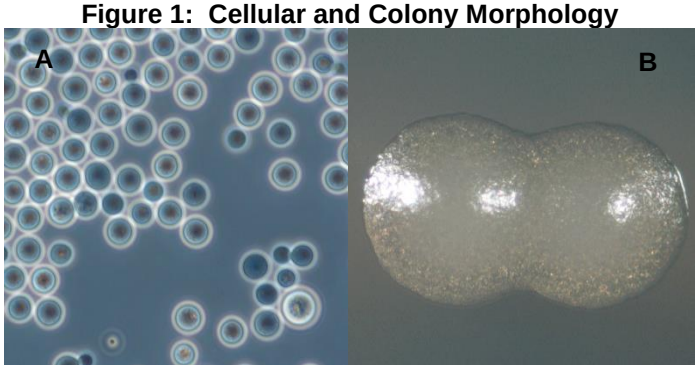
Figure 1: Cellular and Colony Morphology



Date: 26 APR 2017 **Signature:**

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