Certificate of Analysis for NR-50199

Cryptococcus gattii, Strain Alg268

Catalog No. NR-50199

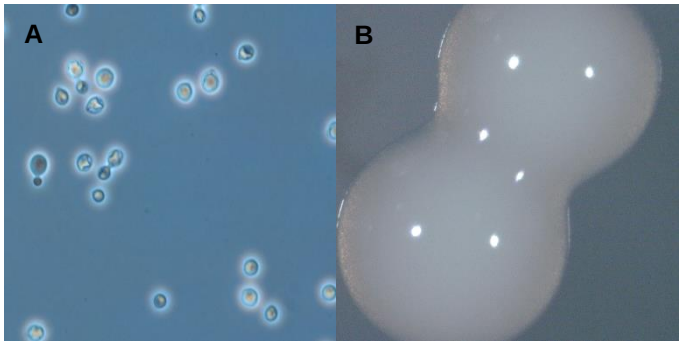
Product Description: *Cryptococcus gattii (C. gattii),* strain Alg268 is a complementation mutant of strain Alg254 (the basidiomycete white collar 2 (BWC2) gene was replaced with a nourseothricin cassette). The wild type BWC2 gene was amplified and inserted into a plasmid, which was transformed into *Agrobacterium tumefaciens*, and transconjugated into *C. gattii*, strain Alg254.

Lot[1]: 64079524 **Manufacturing Date: 11MAR2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology[2]	Report results	Globose to ovoid, single or budding (Figure 1A)
Colony morphology[2]	Report results	Circular, shiny, mucoid and entire (Figure 1B)
Canavanine-glycine-bromthymol blue (CGB) differential medium[3]	Blue (*C. gattii*)	Blue (*C. gattii*)
Genotypic Analysis		
Sequencing of partial 18S rRNA gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 28S rRNA (~ 550 base pairs)	>99% sequence identity to *C. gattii* (GenBank: FJ914888.1)	100% sequence identity to *C. gattii* (GenBank: FJ914888.1)
Sequencing of 26S rRNA gene (620 base pairs)	>99% sequence identity to *C. gattii* (GenBank: KC171326.1)	100% sequence identity to *C. gattii* (GenBank: KC171326.1)
Purity[4]		
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)[2]	Growth	Growth

[1]NR-50199, lot 64079524, was produced by incubation of seed material in modified Sabouraud Dextrose medium and incubated for 4 days at 25°C in an aerobic atmosphere. Yeast were harvested from agar plates with 20% glycerol prior to vialing.
[2]2 days at 25°C in an aerobic atmosphere on modified Sabouraud Dextrose agar
[3]3 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 show yellow to light-green on CGB medium. [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]Clarity of broth was determined by visual inspection after 5 days at 25°C and 37°C in an aerobic atmosphere.

Figure 1: Cellular and Colony Morphology



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Certificate of Analysis for NR-50199

Date: 08 JUL 2016 **Signature:** 

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