Certificate of Analysis for NR-43222

Cryptococcus gattii, Strain Alg254

Catalog No. NR-43222

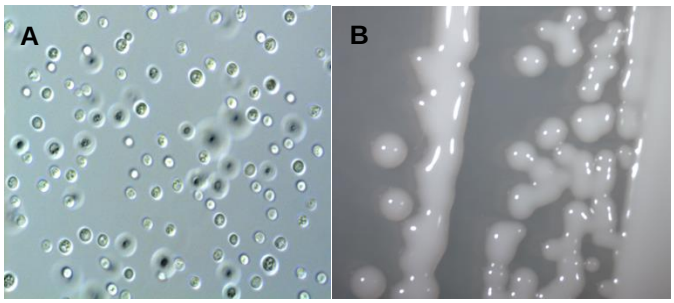
Product Description: *Cryptococcus gattii (C. gattii),* strain Alg254 is a mutant of strain R265 where the basidiomycete white collar 2 (BWC2) gene has been replaced with a cassette conferring resistance to nourseothricin.

Lot[1,2]: 61631755 **Manufacturing Date: 29MAR2013**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology[3]	Report results	Sub-globose to globose, single (Figure 1A)
Colony morphology[3]	Report results	Smooth, mucoid, entire and cream (Figure 1B)
Canavanine-glycine-bromthymol blue (CGB) differential medium[4]	Blue (*C. gatti*)	Blue (*C. gatti*)
Genotypic Analysis		
Sequencing of partial 18S rRNA gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 28S rRNA (~ 520 base pairs)	Consistent with *C. gattii*	Consistent with *C. gattii*[5]
Sequencing of 26S rRNA gene (~ 620 base pairs)	Consistent with *C. gattii*	Consistent with *C. gattii*[5]
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)[2]	Growth	Growth

[1]NR-43222, lot 61631755, was produced by the depositor by incubation at 30°C in Yeast Peptone Dextrose medium overnight. The resultant growth was mixed with 30% glycerol to a final concentration of 15% and vialed.
[2]Quality control testing was performed at BEI Resources.
[3]2 days at 25°C in an aerobic atmosphere on Yeast Mold agar
[4]35 hours at 27°C in an aerobic atmosphere. CGB medium differentiates *C. gattii* from *C. neoformans* based on the ability of *C. gatti* 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.]
[5]Also consistent with *C. neoformans*
[6]Clarity of broth was determined by visual inspection after 2 days at 25°C and 37°C in an aerobic atmosphere.

Figure 1: Cellular morphology (A) and colony morphology (B)



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Certificate of Analysis for NR-43222

Date: 24 FEB 2015 **Signature:** 

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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