

## **Certificate of Analysis for NR-10272**

## Toxoplasma gondii, GT1-FUDR3.3

## Catalog No. NR-10272

**Product Description:** *Toxoplasma gondii* (*T. gondii*), GT1-FUDR3.3 is a virulent Type I parental strain that was used in a genetic cross with the nonvirulent genotype Type III parental strain CTG.11 ARA-SNF (also referred to as CTG.11 ARA-SYN, CTG.11 ARA-A<sup>R</sup>/SNF<sup>R</sup> and CEP.11 ARA-A<sup>R</sup>/SNF<sup>R</sup>).

Lot<sup>1</sup>: 58792126 Manufacturing Date: 09SEP2009

TEST	SPECIFICATIONS	RESULTS
Genotyping <sup>2,3</sup>		
AK16 locus ( <i>Hinf</i> l digestion)	Consistent with parental Type I strain	Consistent with parental Type I strain
L358 locus (HaelII digestion)	Consistent with parental Type I strain	Consistent with parental Type I strain
Drug susceptibility <sup>4</sup>		
Sinefungin (SNF)	Sensitive	Sensitive
Adenine arabinose (Ara-A)	Sensitive	Sensitive
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL	1.1 x 10 <sup>7</sup> cells/mL
Viability (post-freeze) <sup>5</sup>	Growth	Growth
Sterility (21-day incubation)		
Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	No growth	No growth
Mycoplasma Contamination		
DNA Detection by PCR	None detected	None detected

<sup>&</sup>lt;sup>1</sup>NR-10272 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC<sup>®</sup> CRL-1634™) with cell cultivation medium for parasites (ATCC medium 2222: adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated in 95% air, 5% CO₂ for 3 days at 37°C, until lysis of the host cell monolayer was reached.

**Date:** 18 MAR 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>PCR amplification was performed separately for the two loci AK16 and L358. Where appropriate, samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.

<sup>&</sup>lt;sup>3</sup>Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the *Toxoplasma* Genome Map website (<u>Toxoplasma Genome Map</u>).

<sup>&</sup>lt;sup>4</sup>Sinefungin was used at a concentration of 2.7 x 10<sup>-7</sup> M and ara-A was used at a concentration of 1.3 x 10<sup>-4</sup> M, as described (Sibley, L. D., et al. "Generation of a Restriction Fragment Length Polymorphism Linkage Map for *Toxoplasma gondii.*" <u>Genetics</u> 132 (1992): 1003-1015. PubMed: 1360931).

<sup>&</sup>lt;sup>5</sup>Viable cells and signs of infection were seen after 6 days under cultivation conditions at 37°C.

<sup>&</sup>lt;sup>6</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.