

## Certificate of Analysis for NR-10242

## Toxoplasma gondii, Clone c285-4

## Catalog No. NR-10242

**Product Description:** *Toxoplasma gondii* (*T. gondii*), clone c285-4 is a recombinant F1 clone of intermediate virulence selected from progeny of two parallel genetic crosses between a highly virulent Type I parental strain, GT1-FUDR3.3; and the non-virulent Type III parental strain, CTG.11 ARA-SNF.

Lot<sup>1</sup>: 58591157 Manufacturing Date: 15MAY2009

TEST	SPECIFICATIONS	RESULTS
Genotyping <sup>2,3</sup>		
AK16 locus (Hinfl digestion)	Consistent with parental Type III strain	Consistent with parental Type III strain
L358 locus (HaelII digestion)	Consistent with parental Type I strain	Consistent with parental Type I strain
Drug susceptibility <sup>4</sup>		
Sinefungin (SNF)	Resistant	Resistant
Adenine arabinoside (Ara-A)	Resistant	Resistant
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL	3.8 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
Brain Heart Infusion, 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
Mycoplasma Contamination		
DNA Detection by PCR	None detected	None detected

<sup>&</sup>lt;sup>1</sup>NR-10242 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 7 days at 37°C, until lysis of the host cell monolayer was reached.

**Date:** 09 AUG 2012

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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

Biodefense and Emerging Infections Research Resources Repository P.O. Box 4137

Manassas, VA 20108-4137 USA

www.beiresources.org

Fax: 703-365-2898

E-mail: contact@beiresources.org

800-359-7370

<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 (Toxoplasma Genome Map).

<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 10 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.