

## **Certificate of Analysis for NR-10157**

## Toxoplasma gondii, Clone CL13

## Catalog No. NR-10157

**Product Description:** Toxoplasma gondii, clone CL13 is a recombinant F1 clone from progeny of two parallel genetic crosses between a Type II parental strain [ME49 (B7 clone)] and a Type III parental strain (CTG ARA-SYN).

Lot1: 58242546 Manufacturing Date: 03JUL2008

TEST	SPECIFICATIONS	RESULTS
Genotyping <sup>2</sup>		
850 locus (Sfa NI digestion) <sup>3</sup>	Consistent with parental Type II strain	Consistent with parental Type II strain
SAG1 locus <sup>4</sup>	Consistent with parental Type II strain	Consistent with parental Type II strain
Drug susceptibility <sup>5</sup>		
Sinefungin	Resistant	Resistant
Ara-A	Susceptible	Susceptible
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL	2.8 x 10 <sup>7</sup> cells/mL
Viability (post-freeze) <sup>6</sup>	Growth	Growth
Sterility (21-day incubation)		
Harpo's HTYE broth <sup>7</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

¹NR-10157 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC® 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% CO<sub>2</sub> for 14 days at 37°C, until lysis of the host cell monolayer was reached.

**Date: 15 OCT 2009** Signature:

> 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 850 and SAG1. 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 (http://toxomap.wustl.edu/Toxo\_Genetic\_Map\_Table.html).

<sup>&</sup>lt;sup>4</sup>Primer sequences and conditions for PCR are available upon request.

<sup>&</sup>lt;sup>5</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." Genetics 132 (1992): 1003-1015. PubMed: 1360931.)

<sup>&</sup>lt;sup>6</sup>Incubated under cultivation conditions for 2-3 weeks at 37°C.

<sup>&</sup>lt;sup>7</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.