SUPPORTING INFECTIOUS DISEASE RESEARCH

## Toxoplasma gondii, Clone C11 AF

### Catalog No. NR-10245

**Product Description:** *Toxoplasma gondii*, C11 AF is a virulent recombinant F1 clone selected from progeny of two parallel genetic crosses between a highly virulent Type I parental strain,GT1-FUDR3.3 and a less virulent Type III parental strain, CTG.11 ARA-SNF.

### Lot<sup>1</sup>: 58638995

### Manufacturing Date: 10JUL2009

TEST	SPECIFICATIONS	RESULTS
Genotyping <sup>2,3</sup>		
AK16 locus (Hinfl digestion)	Consistent with parental Type I strain	Consistent with parental Type I strain
L358 locus (HaellI digestion)	Consistent with parental Type III strain	Consistent with parental Type III strain
Drug susceptibility <sup>4</sup>		
Sinefungin (SNF)	Resistant	Resistant
Adenine arabinose (Ara-A)	Resistant	Resistant
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL	5.2 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>1</sup>NR-10245 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC<sup>®</sup> CRL-1634<sup>™</sup>) with cell cultivation medium for parasites (<u>ATCC medium 2222</u>: adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated in 95% air, 5% CO₂ for 4 days at 37°C, until lysis of the host cell monolayer was reached.

<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>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>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>5</sup>Viable cells and signs of infection were seen after 9 days under cultivation conditions at 37°C.

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

Date:27 OCT 2009

# Signature: Signature on File

# Title: Technical Manager, BEI Authentication or designee

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800-359-7370 Fax: 703-365-2898 E-mail: contact@beiresources.org