

**Toxoplasma gondii, Clone D9 AF**

**Catalog No. NR-10247**

**Product Description:** *Toxoplasma gondii*, D9 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>: 59609280**

**Manufacturing Date: 30NOV2010**

TEST	SPECIFICATIONS	RESULTS
<b>Genotyping<sup>2,3</sup></b> AK16 locus ( <i>Hinf</i> I digestion) L358 locus ( <i>Hae</i> III digestion)	Consistent with parental Type I strain Consistent with parental Type III strain	Consistent with parental Type I strain Consistent with parental Type III strain
<b>Drug susceptibility<sup>4</sup></b> Sinefungin (SNF) Adenine arabinose (Ara-A)	Resistant Resistant	Resistant Resistant
<b>Viable Cell Count by Hemacytometry (pre-freeze)</b>	> 10 <sup>6</sup> cells/mL	2.3 x 10 <sup>7</sup> cells/mL
<b>Viability (post-freeze)<sup>5</sup></b>	Growth	Growth
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> DNA Detection by PCR	None detected	None detected

<sup>1</sup>NR-10247 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 (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 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 ([Toxoplasma Genome Map](#)).

<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*." *Genetics* 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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Date:** 07 JUN 2011

**Signature:**



**Title:**

Technical Manager, BEI Authentication or designee

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