

## **Certificate of Analysis for NR-49182**

#### Toxoplasma gondii, Strain SF9

### Catalog No. NR-49182

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**Product Description:** *Toxoplasma gondii* (*T. gondii*), strain SF9 is a recombinant F1 clone selected from progeny of a genetic cross between a sinfungin-resistant line of the highly virulent Type I GT-1 strain (GT1-SNF<sup>R</sup>) and a 5-fluoro-2'-deoxyuridine-resistant line of the non-virulent Type 2 ME49 strain (ME49 FUDR<sup>R</sup>).

Lot<sup>1,2</sup>: 63626659 Manufacturing Date: 27JUL2015

TEST	SPECIFICATIONS	RESULTS  Refractile and crescent shaped		
Cell Morphology	Report results			
PCR Assay of Extracted DNA <sup>3,4</sup> AK56 locus	~ 520 base pair amplicon	~ 520 base pair amplicon		
<b>Genotypic Analysis</b> <sup>3,4</sup> Sequencing of AK56 locus (~ 480 base pairs) AK56 locus ( <i>Mfel</i> digestion)	Consistent with <i>T. gondii</i> Consistent with parental Type I strain	Consistent with <i>T. gondii</i> (Figure 1) Consistent with parental Type I strain  8.8 × 10 <sup>7</sup> cells/mL  Viable parasites		
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 <sup>6</sup> cells/mL			
Viability (post-freeze) <sup>5</sup>	Viable parasites			
Sterility (21-day incubation) Harpo's HTYE broth <sup>6</sup> , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic 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-49182 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<sup>®</sup> medium 2222: adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated 6 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

#### Figure 1: AK56 (Chromosome II) Amplicon Sequence

ATTAGGTTTT	TCCGTGTTTT	CGCGGAGTCG	TCTGAGCTCG	GCACTCGCTG	CTTTCCAAAA	TCTCGTTTCA	ACGTATCGCG	
GCGCCGTCAC	CGCGCGCAAT	CCACTGTGAT	GCATGATTCT	GTTTCTAAAA	ACTGCGCATT	TTAGCCGGCT	CGTTTTTGCA	
TACGTTTGGA	CCATAAAACC	TCGTATTGTT	GAAGAAGAAT	GCAATTGGTG	TCTGTGCTGA	TCACCGTATG	AAAATCGGCG	
TGTCTCGCCC	CCTGCCGTGT	GCGCGTCCGC	TTTTTGCGAC	CCCGGTACAC	CCGTTTTTTG	TGGTCAGCGA	GGAACGCACT	
TTTGCTGTTA	TTGTTCACTT	TTCAGCGTAA	CACTGACCCC	TTTCATCGTG	GCAGGAAACG	AACTCTCAGC	AAGAATTTTC	
GAGCACTACT	GCGTCGCAGC	AGCCTAGTGG	GGTGGACACG	CATGTGCAGG	ACGGACAGAA	ACTGCAAGCT	TGTTCC	

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<sup>&</sup>lt;sup>2</sup>Quality control testing completed on post-freeze material unless specified as pre-freeze.

<sup>&</sup>lt;sup>3</sup>PCR amplification of the AK56 locus was performed. Samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis. <sup>4</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>5</sup>Viable cells and signs of infection were seen after 9 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.



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**Date:** 26 JAN 2016

Signature:

**BEI Resources Authentication** 

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

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