

Certificate of Analysis for NR-49199

Toxoplasma gondii, Strain SF61

Catalog No. NR-49199

Product Description: *Toxoplasma gondii* (*T. gondii*), strain SF61 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^R) and a 5-fluoro-2'-deoxyuridine-resistant line of the non-virulent Type II ME49 strain (ME49 FUDR^R).

Lot^{1,2}: 64312880 Manufacturing Date: 24MAY2016

TEST	SPECIFICATIONS	RESULTS		
Cell Morphology	Report results	Refractile and oval-shaped		
PCR Assay of Extracted DNA ^{3,4} AK56 locus	~ 520 base pair amplicon	~ 520 base pair amplicon		
Genotypic Analysis ^{3,4} Sequencing of AK56 locus (~ 510 base pairs) AK56 locus (<i>Mfel</i> digestion)	Consistent with <i>T. gondii</i> Consistent with parental Type II strain	Consistent with <i>T. gondii</i> (Figure 1) Consistent with parental Type II strain 1.7 × 10 ⁷ cells/mL		
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL			
Viability (post-freeze) ⁵	Viable parasites	Viable parasites		
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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		

¹NR-49199 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 for 4 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

TTGTCCTTTT	CCCCACTGCT	TTTATTAGGT	TTTTCCGTGT	TTTCGCGGAG	TCGTCTGAGC	TCGGCACTCG	CTGCTTTCCA	
AAATCTCGTT	TCAACGTATC	GCGGCGCCGT	CACCGCGCGC	AATCCACTGT	GATGCATGAT	TCTGTTTCTA	AAAACTGCGC	
CTTTTAGCCG	GCTCGTTTTT	GCATACGTTT	GGACCATAAA	ACCTCGTATT	GTTGAAGAAG	AATGCAATTT	GTGTCTGTGC	
TGATCACCGT	ATGAAAATCG	GCGTGTCTCG	CCCCTGCCG	TGTGCGCGTC	CGCTTTTTGC	GACCCCGGTA	CACCCGTTTT	
TTGTGGTCAG	CGAGGAACGC	ACTTTTGCTG	TTATTGTTCA	CTTTTCAGCG	TAACACTGAC	CCCTTTCATC	GTGGCAGGAA	
ACGAACTCTC	AGCAAGAATT	TTCGAGCACT	ACTGCGTCGC	AGCAGCCTAG	TGGGGTGGAC	ACGCATGTGC	AGGACGGACA	
GAAACTGCAA	GCTTGTTCCG	CAGGCTAAAA	CTC					

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²Quality control testing completed on post-freeze material unless specified as pre-freeze.

³PCR amplification of the AK56 locus was performed. Samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis. ⁴Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the *Toxoplasma* Genome Map website (<u>Toxoplasma Genome Map</u>).

⁵Viable cells and signs of infection were seen after 1 day at 37°C in an aerobic atmosphere with 5% CO₂.

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



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Date: 14 DEC 2016

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