

Certificate of Analysis for NR-49193

Toxoplasma gondii, Strain SF42

Catalog No. NR-49193

Product Description: *Toxoplasma gondii* (*T. gondii*), strain SF42 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}: 64079280 Manufacturing Date: 22FEB2016

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 (~ 490 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		
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	5.8 × 10 ⁷ 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-49193 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 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

TTTTATTAGG	TTTTTCCGTG	TTTTCGCGGA	GTCGTCTGAG	CTCGGCACTC	GCTGCTTTCC	AAAATCTCGT	TTCAACGTAT
CGCGGCGCCG	TCACCGCGCG	CAATCCACTG	TGATGCATGA	TTCTGTTTCT	AAAAACTGCG	CATTTTAGCC	GGCTCGTTTT
TGCATACGTT	TGGACCATAA	AACCTCGTAT	TGTTGAAGAA	GAATGCAATT	GGTGTCTGTG	CTGATCACCG	TATGAAAATC
GGCGTGTCTC	GCCCCTGCC	GTGTGCGCGT	CCGCTTTTTG	CGACCCCGGT	ACACCCGTTT	TTTGTGGTCA	GCGAGGAACG
CACTTTTGCT	GTTATTGTTC	ACTTTTCAGC	GTAACACTGA	CCCCTTTCAT	CGTGGCAGGA	AACGAACTCT	CAGCAAGAAT
TTTCGAGCAC	TACTGCGTCG	CAGCAGCCTA	GTGGGGTGGA	CACGCATGTG	CAGGACGGAC	AGAAACTGCA	AGCTTGTTCC
GCAGGCTAAA	ACT						

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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 4 days under cultivation conditions at 37°C.

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: 04 OCT 2016

Signature:

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