

Certificate of Analysis for NR-49194

Toxoplasma gondii, Strain SF44

Catalog No. NR-49194

Product Description: *Toxoplasma gondii* (*T. gondii*), strain SF44 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}: 64168064 Manufacturing Date: 31MAR2016

TEST	SPECIFICATIONS	RESULTS Refractile and oval-shaped		
Cell Morphology	Report results			
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 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	1.3 × 10 ⁸ 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-49194 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

GTCCTTTTCC	CCACTGCTTT	TATTAGGTTT	TTCCGTGTTT	TCGCGGAGTC	GTCTGAGCTC	GGCACTCGCT	GCTTTCCAAA
ATCTCGTTTC	AACGTATCGC	${\tt GGCGCCGTCA}$	CCGCGCGCAA	TCCACTGTGA	TGCATGATTC	TGTTTCTAAA	AACTGCGCAT
TTTAGCCGGC	${\tt TCGTTTTTGC}$	ATACGTTTGG	ACCATAAAAC	CTCGTATTGT	TGAAGAAGAA	TGCAATTGGT	GTCTGTGCTG
ATCACCGTAT	GAAAATCGGC	GTGTCTCGCC	CCCTGCCGTG	TGCGCGTCCG	CTTTTTGCGA	CCCCGGTACA	CCCGTTTTTT
GTGGTCAGCG	AGGAACGCAC	TTTTGCTGTT	ATTGTTCACT	TTTCAGCGTA	ACACTGACCC	CTTTCATCGT	GGCAGGAAAC
GAACTCTCAG	CAAGAATTTT	CGAGCACTAC	TGCGTCGCAG	CAGCCTAGTG	GGGTGGACAC	GCATGTGCAG	GACGGACAGA
AACTGCAAGC	TTGTTCCGCA	GGCTAAAACT	CGC				

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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 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: 07 NOV 2016

Signature:

BEI Resources Authentication

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