Certificate of Analysis for NR-49191

Toxoplasma gondii, Strain SF40

Catalog No. NR-49191

Product Description: *Toxoplasma gondii* (*T. gondii*), strain SF40 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 2 ME49 strain (ME49 FUDR^R).

Lot[1,2]: 64045077 **Manufacturing Date: 17FEB2016**

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 (*Mfe*I digestion)	 Consistent with *T. gondii* Consistent with parental Type I strain	 Consistent with *T. gondii* (Figure 1) Consistent with parental Type I strain
Viable Cell Count by Hemacytometry (pre-freeze)	> 10^6 cells/mL	3.5 × 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 No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination DNA Detection by PCR	 None detected	 None detected

[1]NR-49191 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 6 days at 37°C in an aerobic atmosphere with 5% CO_2 until lysis of the host cell monolayer was reached.
[2]Quality control testing completed on post-freeze material unless specified as pre-freeze.
[3]PCR amplification of the AK56 locus was performed. Samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.
[4]Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the *Toxoplasma* Genome Map website (Toxoplasma Genome Map).
[5]Viable cells and signs of infection were seen after 3 days under cultivation conditions at 37°C.
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: AK56 (Chromosome II) Amplicon Sequence

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TGTCCTTTTT CCCCACTGCT TTTATTAGGT TTTTCCGTGT TTTCGCGGAG TCGTCTGAGC TCGGCACTCG CTGCTTTCCA
AAATCTCGTT TCAACGTATC GCGGCGCCGT CACCGCGCGC AATCCACTGT GATGCATGAT TCTGTTTCTA AAAACTGCGC
ATTTTAGCCG GCTCGTTTTT GCATACGTTT GGACCATAAA ACCTCGTATT GTTGAAGAAG AATGCAATTG GTGTCTGTGC
TGATCACCGT ATGAAAATCG GCGTGTCTCG CCCCCTGCCG 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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Date: 04 AUG 2016 **Signature:** 

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