Certificate of Analysis for NR-49188

Toxoplasma gondii, Strain SF35

Catalog No. NR-49188

Product Description: *Toxoplasma gondii* (*T. gondii*), strain SF35 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]: 63888671 **Manufacturing Date: 23NOV2015**

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 (~ 480 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	1.2 × 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 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-49188 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 5 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

```
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 GCCCCCTGCC GTGTGCGCGT CCGCTTTTTG CGACCCCGGT ACACCCGTTT TTTGTGGTCA GCGAGGAACG
CACTTTTGCT GTTATTGTTC ACTTTTCAGC GTAACACTGA CCCCTTTCAT CGTGGCAGGA AACGAACTCT CAGCAAGAAT
TTTCGAGCAC TACTGCGTCG CAGCAGCCTA GTGGGGTGGA CACGCATGTG CAGGACGGAC AGAAACTGCA AGCTTGTTCC
GCA
```

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Certificate of Analysis for NR-49188

Date: 29 MAR 2016 **Signature:** 

BEI Resources Authentication

ATCC®, 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®'s knowledge.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898