Certificate of Analysis for NR-49179

Toxoplasma gondii, Strain 2C10B5

Catalog No. NR-49179

Product Description: *Toxoplasma gondii* (*T. gondii*), strain 2C10B5 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]: 63594338 **Manufacturing Date: 23JUN2015**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology	Report results	Refractile and crescent 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 (~ 500 base pairs) AK56 locus (*Mfe*I digestion)	Consistent with *T. gondii* Consistent with parental Type II strain	Consistent with *T. gondii* (Figure 1) Consistent with parental Type II strain
Viable Cell Count by Hemacytometry (pre-freeze)	> 10^6 cells/mL	4.0 x 10^7 cells/mL
Viability (post-freeze)[5]	Viable parasites	Viable parasites (Figure 2)
Sterility (21-day incubation) Harpo's HTYE broth[6], 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C and 26°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]Quality control testing completed on post-freeze material unless specified as pre-freeze.
[2]NR-49179 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). After a series of passages, the culture was propagated for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 until lysis of the host cell monolayer was reached.
[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.
[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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CACTGCTTTT ATTAGGTTTT TCCGTGTTTT CGCGGAGTCG TCTGAGCTCG GCACTCGCTG CTTTCCAAAA TCTCGTTTCA
ACGTATCGCG GCGCCGTCAC CGCGCGCAAT CCACTGTGAT GCATGATTCT GTTTCTAAAA ACTGCGCCTT TTAGCCGGCT
CGTTTTTGCA TACGTTTGGA CCATAAAACC TCGTATTGTT GAAGAAGAAT GCAATTTGTG TCTGTGCTGA TCACCGTATG
AAAATCGGCG TGTCTCGCCC CCTGCCGTGT GCGCGTCCGC TTTTTGCGAC CCCGGTACAC CCGTTTTTTG TGGTCAGCGA
GGAACGCACT TTTGCTGTTA TTGTTCACTT TTCAGCGTAA CACTGACCCC TTTCATCGTG GCAGGAAACG AACTCTCAGC
AAGAATTTTC GAGCACTACT GCGTCGCAGC AGCCTAGTGG GGTGGACACG CATGTGCAGG ACGGACAGAA ACTGCAAGCT
TGTTCCGCAG GCTAAAACTC
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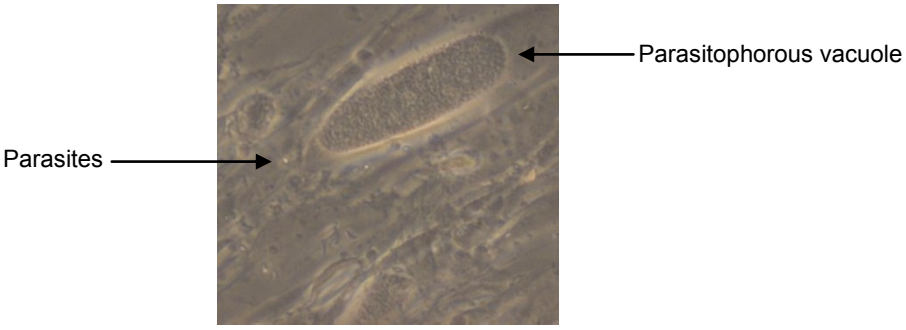
Figure 2: Viable Parasites after 7 days (20x Magnification)



Date: 09 NOV 2015 **Signature:**

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