Certificate of Analysis for NR-49177

Toxoplasma gondii, Strain C7K4

Catalog No. NR-49177

Product Description: *Toxoplasma gondii* (*T. gondii*), strain C7K4 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]: 63516505 **Manufacturing Date: 12JUN2015**

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 (~ 520 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	1.4 x 10^8 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-49177 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 4 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 6 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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CCACTGCTTT TATTAGGTTT TTCCGTGTTT TCGCGGAGTC GTCTGAGCTC GGCACTCGCT GCTTTCCAAA ATCTCGTTTC
AACGTATCGC GGCGCCGTCA CCGCGCGCAA TCCACTGTGA TGCATGATTC TGTTTCTAAA AACTGCGCAT TTTAGCCGGC
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 C
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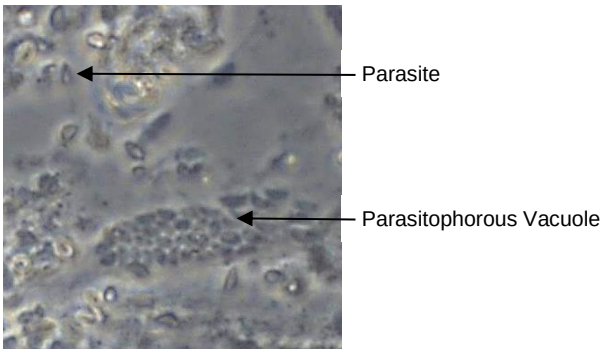
Figure 2: Viable Parasites after 7 Days (40x Magnification)



Date: 09 NOV 2015 **Signature:**

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