SUPPORTING INFECTIOUS DISEASE RESEARCH

Toxoplasma gondii, Strain C7E4

Catalog No. NR-49176

Product Description: *Toxoplasma gondii* (*T. gondii*), strain C7E4 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}: 63500705

Manufacturing Date: 23MAY2015

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 (<i>Mfe</i> l 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	3.2×10^7 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 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

¹Quality control testing completed on post-freeze material unless specified as pre-freeze.

²NR-49176 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% CO₂ until lysis of the host cell monolayer was reached.

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>).

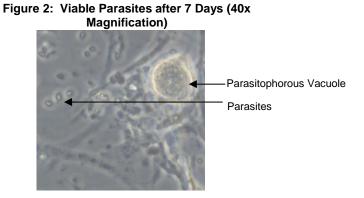
⁵Viable cells and signs of infection were seen after 1 day at 37°C in an aerobic atmosphere with 5% CO₂.

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: AK56 (Chromosome II) Amplicon Sequence

TGCTTTTATT AGGTTTTTCC GTGTTTTCGC GGAGTCGTCT GAGCTCGGCA CTCGCTGCTT TCCAAAATCT CGTTTCAACG TATCGCGGCG CCGTCACCGC GCGCAATCCA CTGTGATGCA TGATTCTGTT TCTAAAAACT GCGCATTTTA GCCGGCTCGT TTTTGCATAC GTTTGGACCA TAAAACCTCG TATTGTTGAA GAAGAATGCA ATTGGTGTCT GTGCTGATCA CCGTATGAAA ATCGGCGTGT CTCGCCCCCT GCCGTGTGCG CGTCCGCTTT TTGCGACCCC GGTACACCCG TTTTTTGTGG TCAGCGAGGA ACGCACTTTT GCTGTTATTG TTCACTTTTC AGCGTAACAC TGACCCCTTT CATCGTGGCA GGAAACGAAC TCTCAGCAAG AATTTTCGAG CACTACTGCG TCGCAGCAGC CTAGTGGGGT GGACACGCAT GTGCAGGACG GACAGAAACT GCAAGCTTGT TCCGCAGGCT AAAACTC **d**i**e**i**i** resources

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Date: 08 JAN 2016

Signature: (

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