b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-20729

Toxoplasma gondii, Strain ME49 (B7 Clone), Genome Sequenced Strain

Catalog No. NR-20729

Product Description: NR-20729 was deposited to BEI Resources as the prototype II isolate that was sequenced as part of the *Toxoplasma gondii* Genome Project at the J. Craig Venter Institute's Genomic Sequencing Center for Infectious Diseases (GSCID). *Toxoplasma gondii (T. gondii)*, strain ME49 (B7 clone) was derived from strain ME49 which was passed singly through a cat and then further cloned by limiting dilution to produce the B7 clone.

Lot[1]: 59907707 **Manufacturing Date: 25APR2011**

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of uracil phosphoribosyltransferase (UPRT) intron 1 (~ 470 bp)	Consistent with *T. gondii,* haplotype II	Consistent with *T. gondii,* haplotype II (Figure 1)
Functional Activity by PCR Amplification[2] UPRT intron 1	~ 560 bp amplicon	~ 560 bp amplicon
Viable Cell Count by Hemacytometry (pre-freeze)	> 10^6 cells/mL	8.7 x 10^7 cells/mL
Viability (post-freeze)[3]	Growth	Growth
Sterility (21-day incubation) Harpo's HTYE broth[4], 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 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]NR-20729 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 in 95% air, 5% CO_2 for 3 days at 37°C, until lysis of the host cell monolayer was reached.
[2]Primer sequences and conditions for PCR are available upon request.
[3]Viable cells and signs of infection were seen after 9 days under cultivation conditions at 37°C.
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: *T. gondii,* Strain ME49 (B7 clone) - UPRT Intron 1 Sequence

GAAGAAAGCA TTCTCCAGGA CATCATCACG AGGTAATCCT TCAACCGAAG TTTGCTTTCC GTGACTCTGC CTGTTGGTTA TACTGCGTGG
CCTTCCCGTC CTGCGGCCCC CTTTCCTCCG CTTGCTGTTT AAATGCTCGT CCTCGTTTTC CTTCCTGCCG CATCCCCGTA TATTTTAAGG
AGAGGGAAAC AGGCGTGAGT TGGACGGCAT GAAAGTTCTC GGCCTGTATG CCGGTTGTGG CGGTCGTTTG CAGATTGCTT TTTTCTTCGA
ATCGGTGCTG TAACCCTCGC GAAGAACGAC GCTGCAAACG ACTTCTCGAA CTCTCAGTCG TGTACTTTAC GTGCTTCCTT TCAGGGACCT
CCCCCCGCGT TACTCATTTG TATTCACAGC TACGAAGTGT CTTGCAAGGT GGATTTCTGC CAGGCTCCAT GTCTCACTCG TTGCGTTTTC
GGAAAAGTTC ATTGTGAACG TTCCCCTTGC GTGTCATGAC TTTATCAGGT TTCCCAATG

Date: 01 SEP 2015 **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.

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