Certificate of Analysis for NR-51146

Toxoplasma gondii, Strain ME49 TIR1-3FLAG

Catalog No. NR-51146

Product Description:

Toxoplasma gondii (*T. gondii*), strain ME49 TIR1-3FLAG was deposited to BEI Resources as a transgenic strain, derived from the Type II luciferase-expressing strain ME49ΔHP::Luc. Strain ME49 TIR1-3FLAG was engineered by replacing the *ku80* gene with the FLAG-tagged auxin receptor TIR1 using CRISPR-Cas9 technology. NR-51146 was produced by cultivation of BEI Resources seed lot 70023321 in human foreskin fibroblast cells (ATCC® CRL-1634™) with Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS). The culture was propagated for 4 days at 37°C in an aerobic atmosphere with 5% CO_2 until lysis of the host cell monolayer was reached.

Lot: 70061938 **Manufacturing Date: 18JUL2024**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 9 days at 37°C in an aerobic atmosphere with 5% CO_2 in DMEM supplemented with 10% HIFBS in human foreskin fibroblast cells (ATCC® CRL-1634™)	Report result	Refractive; crescent-shaped tachyzoites visible
Genotypic Analysis[2] Sequencing of 850 locus (~ 750 base pairs)	≥ 99% sequence identity to *T. gondii*, strain ME49 (GenBank: ABPA02000848.1)	100% sequence identity to *T. gondii*, strain ME49 (GenBank: ABPA02000848.1) (Figure 1)
Phenotypic Analysis FLAG immunofluorescence assay[3]	Positive	Positive (Figure 2)
Viable Cell Count by Hemacytometry[2]	> 10^6 cells per mL	1.1 × 10^8 cells per mL
Viability[1] 9 days at 37°C in an aerobic atmosphere with 5% CO_2 in DMEM supplemented with 10% HIFBS in human foreskin fibroblast cells (ATCC® CRL-1634™)	Growth	Growth
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[4]	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material.
[2]Testing completed on bulk material prior to vialing and freezing.
[3]Immunolabeling of *T. gondii*, strain ME49 TIR1-3FLAG was observed after infection of human skin fibroblast cells (ATCC® CRL-1634™) with NR-51146 lot 70061938 for 3 days.
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

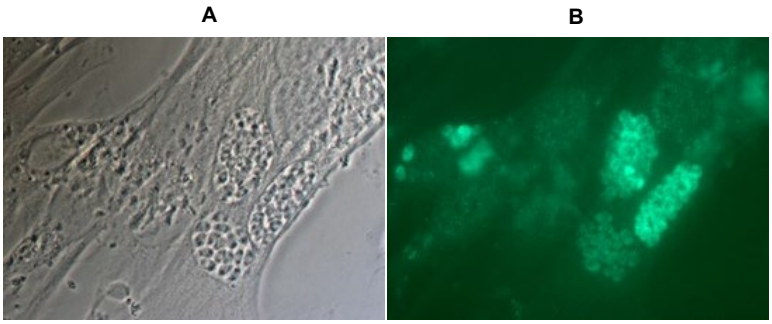
BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Certificate of Analysis for NR-51146

Figure 1: *T. gondii,* ME49 TIR1-3FLAG – 850 Locus Sequence

```
CGATGCTGCA TGGCTGCCAC CCCTTCCTCG TAGCCCCCCT GTCGGTGAGG CAACTGGTCC CCGTGGGGTC TTTGAAAGGC
TCAACCGGGT ACCCGGGCTA CGCGAAGGCG ACCCCCTTCC ACGAGAAGGC CCTCCGACCT TATCGACGCC CGTTCGCCCT
GGCGGCGGCT TGCCATCGCT TCTGGATGTC GGCGCTGCTT TCCCTGGAGG CACCCCTGTT TGTGGGGAGG AAGCAGTAGT
GGTACTAATG GGTGCCTGTG CCTGCCTCCC TCCTTGCTCC GGTGAGCTGC TGCCCCCCAC AGGTCCCTCT TGCTGCATGC
CTCGTGCAGG AGCGCTTGGC GTCGGTGAGT CACCATCTGA CGGTGAAACT GAGCTGTCGC CCAAGCCGCT GCTGCCTGAC
GACGAAGAAG GCCCACCTGA GTGGATTATG ACGACGTCAC CCTCTGGCCC GCTTTCAGAG CCCACGAAAA ATGAAGCAAG
ACGCGGGCCT TGCAGTGGGG ACGGTGATGG CGGCGAACGT TTTCCGGGAA CGTGTGTTCC GATGTCTCTT GTCGGGGATG
TTTCCTTTGG AAGCGAGCCC TTTGCGCCGC AGCACGGACT TTGTGTGTCT GCTGGTACAA GGACAGTCTC TGAAGGACTC
CCCGTTGCAG GTGTGGAATG TAAAGGCCCC TCTACGGGTT TCCCCCCAGA TGCTGGAGAA GGTGGTGA
```

Figure 2: FLAG Immunofluorescence Assay



2A: *T. gondii*-infected human foreskin fibroblast cells (ATCC® CRL-1634™) under bright light.
2B: Immunolabeling of *T. gondii*-infected human skin fibroblast cells using a DYKDDDDK (FLAG) Tag mouse monoclonal antibody (FG4R; Invitrogen™ MA1-91878; 1:500 dilution), followed by Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (Invitrogen® A11001; 1:2000 dilution).

/Sonia Bjoram Brower/
Sonia Bjoram Brower 27 SEP 2024
Technical Manager or designee, ATCC Federal Solutions

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.

Support Provided by NIAID

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