

***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.

Lot¹: 70023320

Manufacturing Date: 19MAR2019

TEST	SPECIFICATIONS	RESULTS
Cell Morphology²	Report results	Refractile
Genotypic Analysis³ Sequencing of 850 locus (~ 760 base pairs)	≥ 99% sequence identity to <i>T. gondii</i> , strain ME49 (GenBank: ABPA02000848.1)	100% sequence identity to <i>T. gondii</i> , strain ME49 (GenBank: ABPA02000848.1) (Figure 1)
PCR Assay of Extracted DNA³ 850 locus ⁴	~ 770 base pair amplicon	~ 770 base pair amplicon
Phenotypic Analysis FLAG immunofluorescence assay ⁵	Positive	Positive (Figure 2)
Viable Cell Count by Hemacytometry³	> 10 ⁶ cells per mL	3.2 x 10 ⁷ cells per mL
Viability (post-freeze)^{2,6}	Viable parasites	Viable parasites
Sterility (21-day incubation)² Harpo's HTYE broth ⁷ , 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, 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 Contamination² DNA Detection by PCR	None detected	None detected

¹NR-51146 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: Dulbecco's Minimal Essential Medium adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated for 4 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

²Testing completed on vial, post-freeze material

³Testing completed on bulk material prior to vialing and freezing

⁴Primer sequences and PCR conditions are available upon request.

⁵Immunolabeling of *T. gondii*, strain ME49 TIR1-3FLAG was observed after infection of human skin fibroblast cells (ATCC® CRL-1634™) with NR-51146 lot 70023320 for 2 days.

⁶Viable cells and signs of infection were seen after 3 days under cultivation conditions at 37°C.

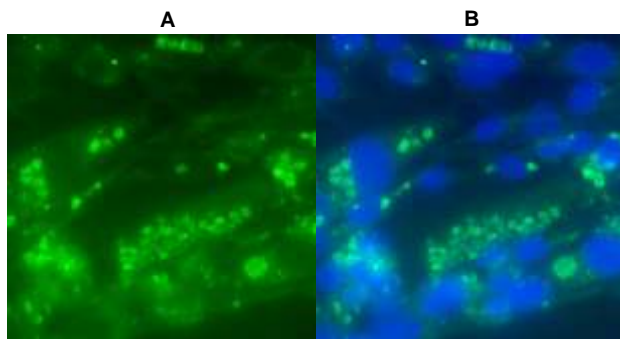
⁷Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: 850 Locus Amplicon Sequence

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TGAGGACCTG GTAACGGCCC TCGTCCAGCC GATGCTGCAT GGCTGCCACC CCTTCCTCGT AGCCCCCCTG
TCGGTGAGGC AACTGGTCCC CGTGGGGTCT TTGAAAGGCT CAACCGGGTA CCCGGGCTAC GCGAAGGCGA
CCCCCTTCCA CGAGAAGGCC CTCCGACCTT ATCGACGCC GTTCGCCCTG GCGGCGGCTT GCCATCGCTT
CTGGATGTCG GCGCTGCTTT CCTTGAGAGC ACCCCTGTTT GTGGGGAGGA AGCAGTAGTG GACTAATGG
GTGCCTGTGC CTGCCTCCCT CCTTGCTCCG GTGAGCTGCT GCGGGGAGGA GGTCCCTCTT GCTGCATGCC
TCGTGCAGGA GCGCTTGGCG TCGGTGAGTC ACCATCTGAC GGTGAAACTG AGCTGTCGCC CAAGCCGCTG
CTGCCTGACG ACGAAGAAGG CCCACCTGAG TGGATTATGA CGACGTCACC CTCTGGCCCG CTTTCAGAGC
CCACGAAAA TGAAGCAAGA CGCGGGCCTT GCAGTGGGGA CGGTGATGGC GGCGAACGTT TTCCGGGAAC
GTGTGTTCCG ATGTCTCTTG TCGGGGATGT TTCCTTTGGA AGCGAGCCCT TTGCGCCGCA GCACGGACTT
TGTGTGCTG CTGGTACAAG GACAGTCTCT GAAGGACTCC CCGTTGCAGG TGTGGAATGT AAAGGCCCTT
CTACGGGTTT CCCCCAGAT GCTGGAGAAG GTGGTGATCT GGAGGCCGAA ACATCC
    
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Figure 2: FLAG Immunofluorescence Assay



2A: Immunolabeling of *T. gondii*-infected human skin fibroblast cells using a DYKDDDDK (FLAG) Tag mouse monoclonal antibody (FG4R; ThermoFisher MA1-91878; 1:200 dilution), followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Invitrogen® A16073; 1:2000 dilution) as secondary antibody.

2B: Image from Figure 2A with overlaid 4',6-diamidino-2-phenylindole (DAPI)-stained image.

/Heather Couch/
Heather Couch

Program Manager or designee, ATCC Federal Solutions

23 MAY 2019

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