

Toxoplasma gondii, Strain RH TIR1-3FLAG

Catalog No. NR-51145

Product Description:

Toxoplasma gondii (*T. gondii*), strain RH TIR1-3FLAG was deposited to BEI Resources as a transgenic strain, derived from the virulent Type I knockout strain RH $\Delta h x g p r t \Delta k u 8 0$, created by transfection with plasmid pTUB1:OsTIR1-3FLAG, SAG1:CAT and selected with chloramphenicol. NR-51145 was produced by cultivation of BEI Resources seed lot 70012764 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 5 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

Lot: 70065848

Manufacturing Date: 30JAN2024

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology ¹ 5 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 (Figure 1)
Genotypic Analysis ² Sequencing of 850 locus (~ 700 base pairs)	≥ 99% sequence identity to <i>T. gondii</i> , strain RH (GenBank: LLKL01000174.1)	100% sequence identity to <i>T. gondii</i> , strain RH (GenBank: LLKL01000174.1) (Figure 2)
Phenotypic Analysis FLAG immunofluorescence assay ³	Positive	Positive (Figure 3)
Viable Cell Count by Hemacytometry ²	> 10 ⁶ cells per mL	4.3 × 10 ⁷ cells/mL
Viability ¹ 5 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 (14-day incubation) ¹ Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 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
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹Testing completed on vial, post-freeze material.

²Testing completed on bulk material prior to vialing and freezing.

³Immunolabeling of *T. gondii*, strain RH TIR1-3FLAG was observed after infection of human skin fibroblast cells (ATCC® CRL-1634™) with NR-51145 lot 70065848 for 2 days.

Figure 1: Cellular Morphology

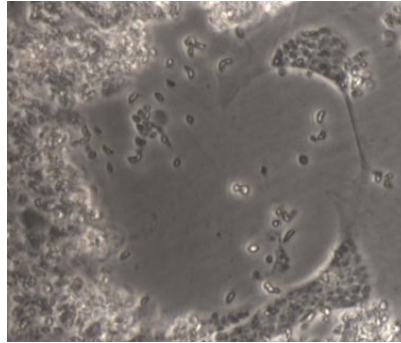
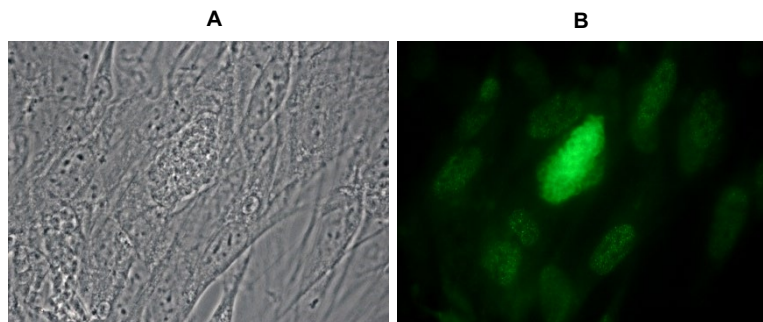


Figure 2: *T. gondii*, Strain RH TIR1-3FLAG – 850 Locus Sequence

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CCTCGTCCAG CCGATGCTGC ATGGCTGCCA CCCCTTCCTC GTAGCCCCCC TGTCGGTGAG GCAACTGGTC CCCGTGGGGT
CTTTAAAAGG CTCAACCGGC TACCGGGGCT ACGCGAAGGC GACCCTCTTC CACGAGAAGG CCCTCCGACC TTATCGACGC
CCGTTCGCC CCGTTCGCC TGGCGACGGC TTGCCATCGC TTCTGGGTGT CGGCGCTGCT TTCCCTGGAG GCATCCCTGT TTGTGGGGAG
GAAGCAGTAG TGGACTAAT GGGTGCCTGT GCCTGCCTCC CTCCTTGCTC CGGTGAGCTG CTGCCCCCA CAGGTCCCTC
TTGCTGCATG CCTCGTGCAG GAGCGCTTGG CGTCGGTGAG TCACCATCTG ACGGTGAAAC TGAGCTGTCG CCCAAGCCGC
TGCTGCCTGA CGACGAAGAA GGCCACCTG AGTGGATTAT GACGACGTCA CCCTCTGGCC CGCCCTCAGA GCCCAGGAAA
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TTTCGGGGAT GTTCCTTTG GAAGCGAGCC CTTTGGCCG CAGCACGGAC TTTGTGTGTC TTTGTGTGTC TGCTGGTACA CGGACAGTCT
CTGAAGGACT CCCCGTTGCA GGTGTGGAAT GTAAAGGCC CTCTACGGGT TTCCCCCA
    
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Figure 3: FLAG Immunofluorescence Assay



- 3A: *T. gondii*-infected human foreskin fibroblast cells (ATCC® CRL-1634™) under bright light.
- 3B: 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 Bjorum Brower/
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Technical Manager or designee, ATCC Federal Solutions

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