

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.

Lot¹: 70012762

Manufacturing Date: 22FEB2018

| TEST | SPECIFICATIONS | RESULTS |
|--|---|--|
| Cell Morphology² | Report results | Crescent-shaped and refractile |
| Genotypic Analysis³ Sequencing of 850 locus (730 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 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 | 2.0 x 10 ⁸ 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-51145 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 for 8 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 RH TIR1-3FLAG was observed using a DYKDDDDK (FLAG) Tag mouse monoclonal antibody (FG4R; ThermoFisher MA1-91878; 1:200 dilution), followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Invitrogen® A16073; 1:2000 dilution) as secondary antibody (Figure 2A) and overlaid with the 4',6-diamidino-2-phenylindole (DAPI)-stained image (Figure 2B).

⁶Viable cells and signs of infection were seen after 4 days under cultivation conditions at 37°C.

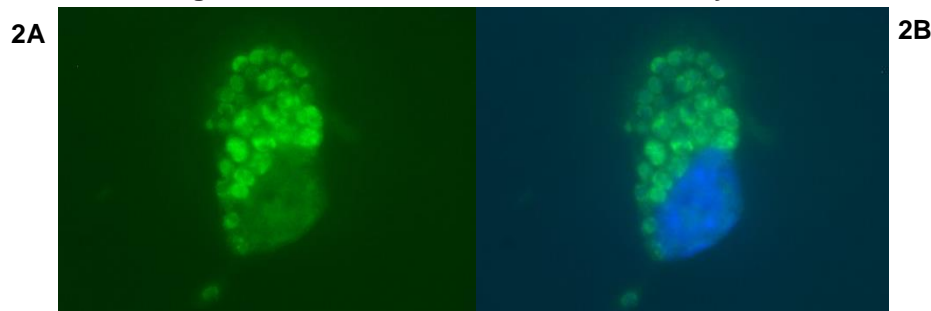
⁷Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: 850 Locus Amplicon Sequence

```

GGCCCTCGTC CAGCCGATGC TGCATGGCTG CCACCCCTTC CTCGTAGCCC CCCTGTGCGT GAGGCAACTG
GTCCCCGTGG GGTCTTTAAA AGGCTCAACC GGCTACCGGG GCTACGCGAA GGCACCCCTC TTCCACGAGA
AGGCCCTCCG ACCTTATCGA CGCCCGTTCG CCCTGGCGAC GGCTTGCCAT CGCTTCTGGG TGTGCGCGCT
GCTTTCCCTG GAGGCATCCC TGTTTGTGGG GAGGAAGCAG TAGTGGCACT AATGGGTGCC TGTGCCTGCC
TCCCTCCTTG CTCCGGTGAG CTGCTGCCCC CCACAGGTCC CTCTTGCTGC ATGCCTCGTG CAGGAGCGCT
TGGCGTCGGT GAGTCACCAT CTGACGGTGA AACTGAGCTG TCGCCCAAGC CGCTGCTGCC TGACGACGAA
GAAGGCCAC CTGAGTGGAT TATGACGACG TCACCCTCTG GCCCGCCCTC AGAGCCCACG AAAAAATGAAG
CAAGACGCGG GCCTTGCAGT GGGGACGGTG ATGGCGGCGA ACGTTTTCCG GGAACGTGTG TTCCGATGTC
GCTTTTCGGG GATGTTTCCT TTGGAAGCGA GCCCTTTGCG CCGCAGCACG GACTTTGTGT GTCTGCTGGT
ACACGGACAG TCTCTGAAGG ACTCCCCGTT GCAGGTGTGG AATGTAAAGG CGCCTCTACG GGTTCCTCCC
CAGATGCTGG AGAAGGTGGT GGTTCGAGG
    
```

Figure 2: FLAG Immunofluorescence Assay



/Heather Couch/
Heather Couch

Program Manager or designee, ATCC Federal Solutions

12 SEP 2018

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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

