

Toxoplasma gondii, Strain Pru A7 Δ hxgprt::gra2-GFP::tub1-FLUC

Catalog No. NR-49335

Product Description:

Toxoplasma gondii (*T. gondii*), strain Pru A7 Δ hxgprt::gra2-GFP::tub1-FLUC was deposited to BEI Resources as a transgenic strain that expresses green fluorescent protein (GFP) and firefly luciferase (FLUC). Strain Pru A7 Δ hxgprt::gra2-GFP::tub1-FLUC is derived from strain Prugniaud (Pru) Δ hxgprt (also referred to as Pru Δ hpt), which lacks the *hxgprt* (hypoxanthine-xanthine-guanine-phosphoribosyltransferase) gene. The parent strain Prugniaud (Pru) is a Type II strain originally isolated in 1964 from a human with lethal congenital toxoplasmosis in Limoges, France. NR-49335 was produced by cultivation of BEI Resources seed lot 64253082 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 3 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

Lot: 70050592

Manufacturing Date: 28MAR2022

TEST	SPECIFICATIONS	RESULTS
Cell Morphology¹ 7 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 Analysis² Sequencing of 850 locus (~ 720 base pairs)	≥ 99% sequence identity to <i>T. gondii</i> , strain Prugniaud (GenBank: GU249506.1)	100% sequence identity to <i>T. gondii</i> , strain Prugniaud (GenBank: GU249506.1)
Confirmation of Genes by PCR Amplification² 850 locus ³ 850 locus (<i>Sfa</i> NI digestion) ³ <i>hxgprt</i> locus ⁴	~ 767 base pair amplicon Consistent with <i>T. gondii</i> Type II No amplicon	~ 767 base pair amplicon Consistent with <i>T. gondii</i> Type II No amplicon
Phenotypic Analysis² Green fluorescent protein (GFP) expression ⁵ Luciferase activity ⁶	Positive Positive	Positive Positive
Viable Cell Count by Hemacytometry²	> 10 ⁶ cells per mL	6.3 × 10 ⁷ cells per mL
Viability¹ 7 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 ⁷ 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 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.

³Primer sequences, annealing temperatures and conditions for restriction enzyme digestion are available at the Toxoplasma Genome Map website (http://toxomap.wustl.edu/Toxo_Genetic_Map_Table.html).

⁴Primer sequences and conditions for PCR are available upon request.

⁵GFP expression was examined by fluorescence microscopy.

⁶Luciferase activity was determined using the Luciferase Assay System (Promega E1500). Parasites were lysed and incubated with luciferase assay reagent. Light was measured using a spectrophotometer with a wavelength of ~ 560 nm.

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

Figure 1: *T. gondii*, Strain Pru A7 Δ hxgprt::gra2-GFP::tub1-FLUC – 850 Locus Sequence

```
CCAGCCGATG CTGCATGGCT GCCACCCCTT CCTCGTAGCC CCCCTGTCGG TGAGGCAACT GGTCCCCGTG GGGTCTTTGA AAGGCTCAAC
CGGGTACCCG GGCTACGCGA AGGCGACCCC CTTCACGAG AAGGCCCTCC GACCTTATCG ACGCCCGTTC GCCCTGGCGG CGGCTTGCCA
TCGCTTCTGG ATGTCGGGCG TGCTTTCCCT GGAGGCACCC CTGTTTGTGG GGAGGAAGCA GTAGTGGTAC TAATGGGTGC CTGTGCCTGC
CTCCCTCCTT GCTCCGGTGA GCTGCTGCCC CCCACAGGTC CCTCTTGCTG CATGCCTCGT GCAGGAGCGC TTGGCGTCGG TGAGTCACCA
TCTGACGGTG AAAC TGAGCT GTCGCCAAG CCGCTGCTGC CTGACGACGA AGAAGGCCCA CCTGAGTGGA TTATGACGAC GTCACCCTCT
GGCCCGCTTT CAGAGCCAC GAAAAATGAA GCAAGACGCG GGCCTTGCAG TGGGGACGGT GATGGCGGCG AACGTTTTCC GGGAACGTGT
GTTCCGATGT CTCTTGTCGG GGATGTTTCC TTTGGAAGCG AGCCCTTTC GCCGCAGCAC GGACTTTGTG TGTCTGCTGG TACAAGGACA
GTCTCTGAAG GACTCCCCGT TGCAGGTGTG GAATGTAAAG GCCCCTCTAC GGGTTTTCCC CCAGATGCTG GAGAAGGTGG TGATCTGGAG
GCCGAAACAT
```

/Sonia Bjoram Brower/
Sonia Bjoram Brower

12 SEP 2023

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.

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.

