Certificate of Analysis for NR-49335

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) was originally isolated in 1964 from a human with lethal congenital toxoplasmosis in Limoges, France. NR-49335 lot 64253081 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 (HIFBS). The culture was propagated for 3 days at 37°C in an aerobic atmosphere with 5% CO_2 until lysis of the host cell monolayer was reached.

Lot: 64253081 **Manufacturing Date: 12MAY2016**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[1] 4 days at 25°C in an aerobic atmosphere with 5% CO_2 in ATCC® medium 2222 supplemented with 10% HIFBS and 10 µg/mL hemin	Report results	Refractile, vacuoles present
Genotypic Analysis[2] Sequencing of 850 locus (~ 710 base pairs) 850 locus (*SfaNI* digestion)	 ≥ 99% sequence identity to *T. gondii*, strain Prugniaud (GenBank: GU249506.1) Consistent with *T. gondii* Type II strain	 100% sequence identity to *T. gondii*, strain Prugniaud (GenBank: GU249506.1) Consistent with *T. gondii* Type II strain
PCR Assay of Extracted DNA[2] 850 locus[3] *hxgprt* locus[4]	 ~ 767 base pair amplicon No amplicon	 ~ 767 base pair amplicon No amplicon
Phenotypic Analysis[2] GFP expression[5] Luciferase activity[6]	 Positive Positive	 Positive Positive
Viable Cell Count by Hemacytometry[2]	> 10^6 cells per mL	8.7 x 10^7 cells/mL
Viability[1] 4 days at 25°C in an aerobic atmosphere with 5% CO_2 in ATCC® medium 2222 supplemented with 10% HIFBS and 10 µg/mL hemin	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[1] 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]Primer 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).
[4]Primer sequences and conditions for PCR are available upon request.
[5]GFP expression was examined by fluorescence microscopy.
[6]Luciferase 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.

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[7]Atlas, 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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GCATGGCTGC CACCCCTTCC TCGTAGCCCC CCTGTCGGTG AGGCAACTGG TCCCCGTGGG GTCTTTGAAA GGCTCAACCG GGTACCCGGG
CTACGCGAAG GCGACCCCCT TCCACGAGAA GGCCCTCCGA CCTTATCGAC GCCCGTTCGC CCTGGCGGCG GCTTGCCATC GCTTCTGGAT
GTCGGCGCTG CTTTCCCTGG AGGCACCCCT GTTTGTGGGG AGGAAGCAGT AGTGGTACTA ATGGGTGCCT GTGCCTGCCT CCCTCCTTGC
TCCGGTGAGC TGCTGCCCCC CACAGGTCCC TCTTGCTGCA TGCCTCGTGC AGGAGCGCTT GGCGTCGGTG AGTCACCATC TGACGGTGAA
ACTGAGCTGT CGCCCAAGCC GCTGCTGCCT GACGACGAAG AAGGCCCACC TGAGTGGATT ATGACGACGT CACCCTCTGG CCCGCTTTCA
GAGCCCACGA AAAATGAAGC AAGACGCGGG CCTTGCAGTG GGGACGGTGA TGGCGGCGAA CGTTTTCCGG GAACGTGTGT TCCGATGTCT
CTTGTCGGGG ATGTTTCCTT TGGAAGCGAG CCCTTTGCGC CGCAGCACGG ACTTTGTGTG TCTGCTGGTA CAAGGACAGT CTCTGAAGGA
CTCCCCGTTG CAGGTGTGGA ATGTAAAGGC CCCTCTACGG GTTTCCCCCC AGATGCTGGA GAAGGTGGTG
ATCTGGAG
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/Heather Couch/
Heather Couch 16 JUL 2020
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