b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-51148

Toxoplasma gondii, Strain RH MIC2-GLuc-C-myc

Catalog No. NR-51148

Product Description: *Toxoplasma gondii (T. gondii)*, strain RH MIC2-GLuc-C-myc was deposited to BEI Resources as a transgenic strain derived from the virulent Type I strain RH, created by co-transfection with pMIC2-GLuc-C-myc and pBS-TUB1CatSAG1 and selected with chloramphenicol.

Lot[1]: 70013168 **Manufacturing Date: 12MAR2018**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[2]	Report results	Crescent-shaped and refractile
Genotypic Analysis[3] Sequencing of 850 locus (~ 710 base pairs) Sequencing of *Gaussia princeps* luciferase (GLuc) gene (~ 510 base pairs)	≥ 99% sequence identity to *T. gondii*, strain RH (GenBank: LLKL01000174.1) ≥ 99% sequence identity to synthetic construct GLuc (GenBank: MF882921.1)	100% sequence identity to *T. gondii*, strain RH (GenBank: LLKL01000174.1) (Figure 1) 100% sequence identity to synthetic construct GLuc (GenBank: MF882921.1) (Figure 2)
PCR Assay of Extracted DNA[3] 850 locus[4] GLuc[5]	 ~ 770 base pair amplicon ~ 530 base pair amplicon	 ~ 770 base pair amplicon ~ 530 base pair amplicon
Phenotypic Analysis C-myc immunofluorescence assay[6]	 Positive	 Positive (Figure 3)
Viable Cell Count by Hemacytometry[3]	> 10^6 cells per mL	1.4 x 10^8 cells per mL
Viability (post-freeze)[2,7]	Viable parasites	Viable parasites
Sterility (21-day incubation)[2] Harpo's HTYE broth[8], 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 Contamination[2] DNA Detection by PCR	 None detected	 None detected

[1]NR-51148 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 3 days at 37°C in an aerobic atmosphere with 5% CO_2 until lysis of the host cell monolayer was reached.
[2]Testing completed on vialed, post-freeze material.
[3]Testing completed on bulk material prior to vialing and freezing.
[4]Primer sequences and PCR conditions are available upon request.
[5]Primer sequences as reported in Brown, K. M., S. Lourido and L. D. Sibley. "Serum Albumin Stimulates Protein Kinase G-dependent Microneme Secretion in *Toxoplasma gondii*." J. Biol. Chem. 291 (2016): 9554-9565. PubMed: 26933037.
[6]Immunolabeling of *T. gondii*, strain RH MIC2-GLuc-C-myc was observed using a C-myc tag (Myc.A7) mouse monoclonal antibody (ThermoFisher, MA1-21316; 1:1000 dilution), followed by fluorescein isothiocyanate (FITC)-congugated goat anti-mouse IgG (Invitrogen® A16073; 1:2000 dilution) as secondary antibody (Figure 3A) and overlaid with the 4′,6-diamidino-2-phenylindole (DAPI)-stained image (Figure 3B).
[7]Viable cells and signs of infection were seen after 4 days under cultivation conditions at 37°C.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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Figure 1: 850 Locus Amplicon Sequence

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CCTCGTCCAG CCGATGCTGC ATGGCTGCCA CCCCTTCCTC GTAGCCCCCC TGTCGGTGAG GCAACTGGTC
CCCGTGGGGT CTTTAAAAGG CTCAACCGGC TACCGGGGCT ACGCGAAGGC GACCCTCTTC CACGAGAAGG
CCCTCCGACC TTATCGACGC CCGTTCGCCC TGGCGACGGC TTGCCATCGC TTCTGGGTGT CGGCGCTGCT
TTCCCTGGAG GCATCCCTGT TTGTGGGGAG GAAGCAGTAG TGGCACTAAT GGGTGCCTGT GCCTGCCTCC
CTCCTTGCTC CGGTGAGCTG CTGCCCCCCA CAGGTCCCTC TTGCTGCATG CCTCGTGCAG GAGCGCTTGG
CGTCGGTGAG TCACCATCTG ACGGTGAAAC TGAGCTGTCG CCCAAGCCGC TGCTGCCTGA CGACGAAGAA
GGCCCACCTG AGTGGATTAT GACGACGTCA CCCTCTGGCC CGCCCTCAGA GCCCACGAAA AATGAAGCAA
GACGCGGGCC TTGCAGTGGG GACGGTGATG GCGGCGAACG TTTTCCGGGA ACGTGTGTTC CGATGTCGCT
TTTCGGGGAT GTTTCCTTTG GAAGCGAGCC CTTTGCGCCG CAGCACGGAC TTTGTGTGTC TGCTGGTACA
CGGACAGTCT CTGAAGGACT CCCCGTTGCA GGTGTGGAAT GTAAAGGCGC CTCTACGGGT TTCCCCCCAG
ATGCTGGAGA AGG
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Figure 2: GLuc Gene Amplicon Sequence

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GAAGCCCACC GAGAACAACG AAGACTTCAA CATCGTGGCC GTGGCCAGCA ACTTCGCGAC CACGGATCTC
GATGCTGACC GCGGGAAGTT GCCCGGCAAG AAGCTGCCGC TGGAGGTGCT CAAAGAGATG GAAGCCAATG
CCCGGAAAGC TGGCTGCACC AGGGGCTGTC TGATCTGCCT GTCCCACATC AAGTGCACGC CCAAGATGAA
GAAGTTCATC CCAGGACGCT GCCACACCTA CGAAGGCGAC AAAGAGTCCG CACAGGGCGG CATAGGCGAG
GCGATCGTCG ACATTCCTGA GATTCCTGGG TTCAAGGACT TGGAGCCCAT GGAGCAGTTC ATCGCACAGG
TCGATCTGTG TGTGGACTGC ACAACTGGCT GCCTCAAAGG GCTTGCCAAC GTGCAGTGTT CTGACCTGCT
CAAGAAGTGG CTGCCGCAAC GCTGTGCGAC CTTTGCCAGC AAGATCCAGG GCCAGGTGGA CAAGATCAAG
GGGGCCGGTG GTGACC
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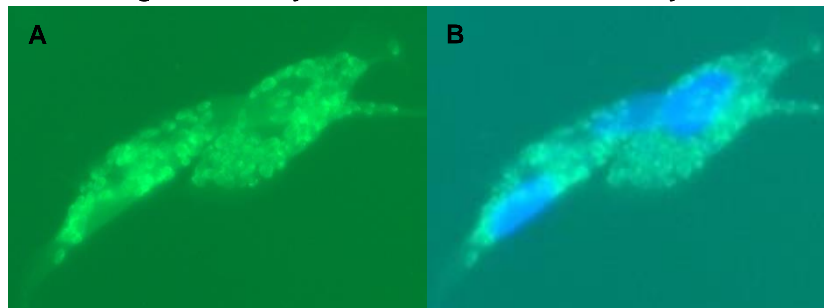
Figure 3: C-myc Immunofluorescence Assay



/Heather Couch/
Heather Couch — 27 AUG 2018
Program Manager or designee, ATCC Federal Solutions

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