

***Toxoplasma gondii*, Strain RH GCaMP6f**

Catalog No. NR-51149

Product Description: *Toxoplasma gondii* (*T. gondii*), strain RH GCaMP6f was deposited to BEI Resources as a transgenic strain derived from the virulent Type I strain RH created by transfection with ptub-GCaMP6(x)/sagCAT and selected with chloramphenicol. *T. gondii*, strain RH GCaMP6f is reported to express a derivatized variant of the Green Fluorescent Protein (GFP), GCaMP6f, whose fluorescence is directly proportional to calcium (Ca²⁺) concentration.

Lot¹: 70013170

Manufacturing Date: 09MAR2018

TEST	SPECIFICATIONS	RESULTS
Cell Morphology²	Report results	Refractile, vacuoles present
Genotyping³ Sequencing of 850 locus (~ 740 base pairs) Sequencing of GCaMP6 locus (~ 1150 base pairs)	≥ 99% sequence identity to <i>T. gondii</i> , strain RH (GenBank: GU249505) ≥ 99% sequence identity to synthetic construct GCaMP6 (GenBank: KP230401)	100% sequence identity to <i>T. gondii</i> , strain RH (GenBank: GU249505) (Figure 1) 99.0% sequence identity to synthetic construct GCaMP6 (GenBank: KP230401) (Figure 2) ⁴
Confirmation of Genes by PCR Amplification^{3,5} 850 locus GCaMP6 locus	~ 770 base pair amplicon ~ 1380 base pair amplicon	~ 770 base pair amplicon ~ 1380 base pair amplicon
Viable Cell Count by Hemacytometry³	> 10 ⁶ cells/mL	8.1 x 10 ⁷ cells/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-51149 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: DMEM supplemented with 10% heat-inactivated fetal bovine serum). The culture was propagated for 4 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

²Testing completed on vialled, post-freeze material.

³Testing completed on bulk material prior to vialing and freezing.

⁴Also showed consistent with synthetic construct GCaMP3 (GenBank: HM143847)

⁵Primer sequences and conditions for PCR are available upon request.

⁶Viable cells and signs of infection were seen after 3 days at 37°C in an aerobic atmosphere with 5% CO₂ in DMEM supplemented with 10% heat-inactivated fetal bovine serum.

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

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GGCCCTCGTC CAGCCGATGC TGCATGGCTG CCACCCCTTC CTCGTAGCCC CCCTGTCGGT GAGGCAACTG GTCCCCGTGG
GGTCTTTAAA AGGCTCAACC GGCTACCGGG GCTACGCGAA GGCACCCCTC TTCCACGAGA AGGCCCTCCG ACCTTATCGA
CGCCCGTTTC CCCTGGCGAC GGCTTGCCAT CGCTTCTGGG TGTCGGCGCT 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
AAAAATGAAG CAAGACGCGG GCCTTGCACT GGGGACGGTG ATGGCGGCGA ACGTTTTCCG GGAACGTGTG TTCCGATGTC
GCTTTTCGGG GATGTTTCCT TTGGAAGCGA GCCCTTTGCG CCGCAGCACG GACTTTGTGT GTCTGCTGGT ACACGGACAG
TCTCTGAAGG ACTCCCCGTT GCAGGTGTGG AATGTAAAGG CGCCTCTACG GGTTCCTCCC CAGATGCTGG AGAAGGTGGT
GGTTTGGAGG CCGAAACATC
    
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Figure 2: GCaMP6 Locus Amplicon Sequence

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GCAGTCAGAG CTATAGGTCG GCTGAGCTCA CTCGAGAACG TCTATATCAA GGCCGACAAG CAGAAGAACG GCATCAAGGC
GAACTTCAAG ATCCGCCACA ACATCGAGGA CGGCGGCGTG CAGCTCGCCT ACCACTACCA GCAGAACACC CCCATCGGCG
ACGGCCCCGT GCTGCTGCCC GACAACCACT ACCTGAGCGT GCAGTCCAAA CTTTCGAAAG ACCCCAACGA GAAGCGCGAT
CACATGGTCC TGCTGGAGTT CGTGACCGCC GCCGGGATCA CTCTCGGCAT GGACGAGCTG TACAAGGGCG GTACCGGAGG
GAGCATGGTG AGCAAGGGCG AGGAGCTGTT CACCGGGGTG GTGCCCATCC TGGTCGAGCT GGACGGCGAC GTAAACGGCC
ACAAGTTCAG CGTGTCCGGC GAGGGTGAGG GCGATGCCAC CTACGGCAAG CTGACCCTGA AGTTCATCTG CACCACCGGC
AAGCTGCCCC TGCCCTGGCC CACCCTCGTG ACCACCCTGA CCTACGGCGT GCAGTGCTTC AGCCGCTACC CCGACCACAT
GAAGCAGCAC GACTTCTTCA AGTCCGCCAT GCCCGAAGG TACATCCAGG AGCGCACCAT CTTCTTCAAG GACGACGGCA
ACTACAAGAC CCGCGCCGAG GTGAAGTTCG AGGGCGACAC CCTGGTGAAC CGCATCGAGC TGAAGGGCAT CGACTTCAAG
GAGGACGGCA ACATCCTGGG GCACAAGCTG GAGTACAACC TGCCGGACCA ACTGACTGAA GAGCAGATCG CAGAATTTAA
AGAGGAATTC TCCCTATTTG ACAAGGACGG GGATGGGACA ATAACAACCA AGGAGCTGGG GACGGTGATG CGGTCTCTGG
GGCAGAACCC CACAGAAGCA GAGCTGCAGG ACATGATCAA TGAAGTAGAT GCCGACGGTG ACGGCACAAT CGACTTCCCT
GAGTTCCTGA CAATGATGGC AAGAAAAATG AAATACAGGG ACACGGAAAG AGAAAATTAGA GAAGCGTTTC GTGTGTTTGA
TAAGGATGGC AATGGCTACA TCAGTGCAGC AGAGCTTCGC CACGTGATGA CAAACCTTGG AGAGAAGTTA ACAGATGAAG
AGGTTGATGA AATGATCAGG GAAGCAGACA TCGA
    
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13 JUN 2018

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