

***Toxoplasma gondii*, Strain RH Δ rop16 + rop16**

Catalog No. NR-49334

Product Description: *Toxoplasma gondii* (*T. gondii*), strain RH Δ rop16 + rop16 is a complementation mutant of a Δ rop16 deletion mutant created from the virulent Type I strain RH by replacing the rop16 locus with an HXGPRT cassette followed by transfection with a plasmid containing HA-tagged Type I rop16 and the bleomycin resistance gene. Complementation mutants were selected for bleomycin resistance and single-cell cloning by limited dilution.

Lot^{1,2}: 63721677

Manufacturing Date: 21SEP2015

TEST	SPECIFICATIONS	RESULTS
Cell Morphology	Report results	Refractile and crescent shaped
PCR Assay of Extracted DNA^{3,4} ROP5 locus ROP16 locus ROP18 locus	~ 887 base pair amplicon ~ 986 base pair amplicon ~ 804 base pair amplicon	~ 887 base pair amplicon ~ 986 base pair amplicon ~ 804 base pair amplicon
Genotypic Analysis^{3,4} Sequencing of ROP5 locus (~ 760 base pairs) Sequencing of ROP16 locus (~ 890 base pairs) Sequencing of ROP18 locus (~ 700 base pairs)	Consistent with <i>T. gondii</i> Consistent with <i>T. gondii</i> Consistent with <i>T. gondii</i>	Consistent with <i>T. gondii</i> (Figure 1) Consistent with <i>T. gondii</i> (Figure 1) Consistent with <i>T. gondii</i> (Figure 1)
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	4.8 × 10 ⁷ cells/mL
Viability (post-freeze)⁵	Viable parasites	Viable parasites
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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 DNA Detection by PCR	None detected	None detected

¹NR-49334 was produced from a frozen subculture of the deposited material. The subculture was cultivated 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% CO₂ until lysis of the host cell monolayer was reached.

²Quality control testing completed on post-freeze material unless specified as pre-freeze.

³PCR amplification was performed separately for the three loci ROP5, ROP16 and ROP18. Where appropriate, samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.

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

⁵Viable cells and signs of infection were seen after 4 days under cultivation conditions at 35°C.

⁶Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: ROP5 Amplicon Sequence

TTCCGTAGGC TGAGGGGCTT CTTCCGGACG CTTACGCCGA GGTGGCTCTC CGGTCTCGGC CGCCGGGCGC AAAGATGGTG
 GAGAGGGAGA CAGAGACCGC TGCTGGACCC TTCGTTTCAT GGGTTGGAAG CTGGAGATTC GTTCATGCGC GACCTGCTGA
 AACGTGAAAA ASAGCTGATT GGATACTGTC GCGAAGAAGC GTTGAAAGAA CCTGCAGCGA TGGTTGAGGC TGTCACGGCA
 ACCGTATGGC CGCAAAATGC TGAAACAACC GTGGATTCCC TTTTGAKTCA GGGAGAGCGG AAGTTGAAAT TGGTGGGGCC
 TCTTCGAGTC GGTGACCGAT CTGTSGTATT TTTAGTAAGG GATGTRGAGC GCYTGGAGRA TTTTCGCTCTG AAGGTYTTCA
 CTATGGGTGC CGAGAATTCC CGATCAGAGC KGGAGCGGTT GCATGAAGYG ACTTTTTCGG CAGCGAGGTT GCTTGGGGAG
 AGTCCAGAGG AGGCACGGGA CAGACGCAGG CTTTTACTTC CYTCCGATGY TGTGGCAGTT CAGTYTCAGC CCCCTTTCGY
 TCAGCTGAGT CCAGGACAGR GCGACTATGC AGTCGCGAAC TATTTGCTTC TCATGCCC GC TGCGTCGGTG GATCTTGAAT
 TGCTCTTTAG SACATTGGAY TTCSTGTATG TAYTCAGGRG KRCMGAAGRT WTTTTAGCGC TTCACATACT AACGGCACAG
 CTGATCCGTC TGGCAGCCAA CCTGCAGAGC AAAGGACT

ROP16 Amplicon Sequence

ATGGTTTCCA ACAGAGTCAA TATATACGCT ATGGAGTCT CCGCCGCAAC GATTGACACA TCGAAAGCCA TCTCTATCTG
 GGGTGGTTCGT TACCGAATTT CAAGAGCCAC AAGAACAGTA TGGCGCAGCG AGCAGTCTTG CGTCCCTCGCC AAAGGGATAC
 GTCGGTGGCG CAAGCTCTAG TGCATTGTCA GGAAAGGCGG TGCCGACGCC TGCGTCGCTT GGTCAAGAAA ATCCTCTTTT
 TCCTGGTCAG AGCGCTACAT TGGATTGAGG AATACAGTCT CCGGCACAAA AGCGTCGGGG ATCCCCTCAA AGACAGAGTG
 CGATGCCGAC CGGAAATCCA GCAGATAGCG GCGCCTCGCA GCTTGCCCTC AGTCATTCTA GTTATGTATC AGTACAAGCT
 TCTCTTGCGA AACGTTTCTG ACGCATCCGG CCGTTCGAC TTTGAGAAGA GGTCTGGAA GAAGTTCAGC AGCTGAAAGC
 AGCTGCCGCA CAGCTTCTCG TAGCGGTTC GACTATGAG GCAATGCGGG CTGTTCTGCA AGAGGCGGTC CTCTCAGAAC
 AGAGGTTGC TGCCCCTAAG CGGAAGAGAA AGCAACCTCC AGGAGCGGTG GAGTCAGCTG TTGACGAAGT GTTCTCTCCA
 AATGAGCGTG TCATGATGAT AAATGCCAAC GGAGTGCCGA TCGTCTATA CAATCGTGGG CACCTCGGCA GTGGACATTT
 CGGGGCTGTC ATCAAGGCCA GCTTAGACGA TGGGACGCTG TATGCAGCGA AGGTGCCGTA CAGCCAGATC GTCCCGAATG
 CTGATGCCAC GTCAGCAGAA CTGGAGGCGG GAATTTCTC AGCTAGGGCG GAGTTGGTAA AGACAATTCG ACAGGAGTTG
 GATGTTTCGGG AT

ROP18 Amplicon Sequence

TTTGCGGGCA CAAAGACGGC GATCTGAATT GGTTTTTGAG AAGGCGGATT CTGGATGCGT CATCGGCAAA CGCATCCTGG
 CGCACATGCA AGAACAAATC GGGCAGCCTC AAGCGCTAGA AAATAGTGAA CGACTGGATA GAATTCTGAC TGTCGCCGCC
 TGGCCTCCGG ACGTTCCAAA AAGATTTGTT TCTGTGACTA CCGGTGAAAC CCGGACGCTG GTGAGAGGTG CACCCCTTGG
 CTCTGGTGGG TTCGCCACTG TATATGAGGC TACAGACGTG GAGACGAATG AAGAGTTGGC TGTTAAGGTT TTCATGTGAG
 AAAAGGAGCC CACCGATGAG ACTATGCTTG ACTTGCAGAG GGAGTCGTCC TGCTACAGGA ACTTTAGTCT AGCCAAGACG
 GCGAAGGATG CCCAGGAAAG CTGTAGATTG ATGGTTCTTA GTGATGTTGT GATGTTAGAG GGACAGCCAG CATCCACAGA
 GGTGCGTATT GGTTTGACGA CTCGGTGGGT ACCAAACTAT TTTCTTCTCA TGATGCGGGC AGAAGCGGAC ATGAGCAAAG
 TCATTTTCATG GGTATTTGGA GATGCGTCTG TCAATAAAAAG TGAATTTGGC CTGGTCGTTT GAATGTACCT ATCCAGTCAG
 GCAATCAAAC TAGTGGCCAA TGTTCAAGCT CAGGGAATTG TGCATACGGA TATCAAACCG G

Date: 25 APR 2016

Signature: 

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