

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

Catalog No. NR-49333

Product Description: *Toxoplasma gondii* (*T. gondii*), strain RH Δ rop16 was deposited to BEI Resources as a mutant of the virulent Type I strain RH created by the deletion of the *rop16* locus with a targeting construct containing an HXGPRT marker flanked by ~ 2 kilobase of the *rop16* upstream and downstream genomic regions.

Lot^{1,2}: 63721676

Manufacturing Date: 31AUG2015

TEST	SPECIFICATIONS	RESULTS
Cell Morphology	Report results	Refractile and crescent shaped
PCR Assay of Extracted DNA^{3,4} ROP5 locus ROP16 locus ROP18 locus	~ 900 base pair amplicon No amplicon ~ 800 base pair amplicon	~ 900 base pair amplicon No amplicon ~ 800 base pair amplicon
Genotypic Analysis^{3,4} Sequencing of ROP5 locus (720 base pairs) Sequencing of ROP18 locus (~ 710 base pairs)	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)
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	5.4 x 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

¹Quality control testing completed on post-freeze material unless specified as pre-freeze.

²NR-49333 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 4 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

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 3 days under cultivation conditions at 37°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

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CTGTGCCGTT AGTATGTGAA GCGCTAAAAAT ATCTTCTCCA CCCCTGARTA CATAACAGGAA ATCCAATGTG CTAAAGAGCA
ATTCAAGATC CACCGACGCA GCGGGCATGA GAAGCAAATA GTTCGCGACT GCATAGTCGC TCTGTCTGG ACTCAGCTGA
GCGAAAGGGG GCTGAGACTG AACTGCCACA GCATCGGAGG GAAGTAAAAG CCTGCGTCTG TCCCGTGCCT CCTCTGGACT
CTCCCCAAGC AACCTCGCTG CCGCAAAAGT CGCTTCATGC AACCGCTCCA GCTCTGATCG GGAATTCTCG GCACCCATAG
TGAAAACCTT CAGAGMGAAG TCCTCCAGGC GCTCTACATC CCTTACTAAA AATACGACAG ATCGGTCACC GACTCGAAGA
GGCTCCACCA ATTTCAACTT CCGCTCTCCC TGAATCAAAA GKGAATCCAC GGTTGTTTCA GCATTTTTCG GCCATACAGT
TGCCGTGACA GCCTCAACCA TCGCTGCAGG TTCTTTCAAC GCTTCTTCGC GACAGTATCC AATCAGCTCT TYTTTACGTT
TCAGCAGGTC GCGCATGAAC GAATCTCCAG CTTCCAACCC ATGAAACGAA GGGTCCAGCA GCGGTCTCTG TCTCCCTCTC
CACCATCTTT GCGCCC GGCG GCGGAGACCG GAGAGCCACC TCGGCGTAAG GCGTCCGAAG AAGCCCCTCA GCCTACGGAA
    
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ROP18 Amplicon Sequence

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TTTGCGGGCA CAAAGACGGC GATCTGAATT GGTTTTGAG 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 TTCATGTCAG
AAAAGGAGCC CACCGATGAG ACTATGCTTG ACTTGCAGAG GGAGTCGTCC TGCTACAGGA ACTTTAGTCT AGCCAAGACG
GCGAAGGATG CCCAGGAAAG CTGTAGATTC ATGGTTCCTA GTGATGTTGT GATGTTAGAG GGACAGCCAG CATCCACAGA
GGTCGTGATT GGTTTGACGA CTCGGTGGGT ACCAAACTAT TTTCTTCTCA TGATGCGGGC AGAAGCGGAC ATGAGCAAAG
TCATTTTATG GGTATTTGGA GATGCGTCTG TCAATAAAAAG TGAATTTGGC CTGGTCGTTT GAATGTACCT ATCCAGTCAG
GCAATCAAAC TAGTGGCCAA TGTTCAAGCT CAGGGAATTG TGCATACGGA TATCAAACCG GCGAATTTCC TC
    
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Date: 06 JUN 2016

Signature: 

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