

**Toxoplasma gondii, Strain RH  $\Delta$ rop5**

**Catalog No. NR-49332**

**Product Description:** *Toxoplasma gondii* (*T. gondii*), strain RH  $\Delta$ rop5 was deposited to BEI Resources as a mutant of the virulent Type I strain RH created by replacing the entire *rop5* locus of the double-knockout parental strain RH $\Delta$ ku80 $\Delta$ hxcg with an HXGPRT cassette.

**Lot<sup>1,2</sup>: 63721673**

**Manufacturing Date: 31AUG2015**

TEST	SPECIFICATIONS	RESULTS
<b>Cell Morphology</b>	Report results	Refractile and crescent shaped
<b>PCR Assay of Extracted DNA<sup>3,4</sup></b> ROP5 locus ROP16 locus ROP18 locus	No amplicon ~ 986 base pair amplicon ~ 804 base pair amplicon	No amplicon ~ 986 base pair amplicon ~ 804 base pair amplicon
<b>Genotypic Analysis<sup>3,4</sup></b> Sequencing of ROP16 locus (~ 770 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)
<b>Viable Cell Count by Hemacytometry (pre-freeze)</b>	> 10 <sup>6</sup> cells/mL	1.5 x 10 <sup>7</sup> cells/mL
<b>Viability (post-freeze)<sup>5</sup></b>	Viable parasites	Viable parasites
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>6</sup> , 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
<b>Mycoplasma Contamination</b> DNA Detection by PCR	None detected	None detected

<sup>1</sup>Quality control testing completed on post-freeze material unless specified as pre-freeze.

<sup>2</sup>NR-49332 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% CO<sub>2</sub> until lysis of the host cell monolayer was reached.

<sup>3</sup>PCR 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.

<sup>4</sup>Primer sequences and conditions for PCR are available upon request.

<sup>5</sup>Viable cells and signs of infection were seen after 3 days under cultivation conditions at 37°C.

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

Figure 1: ROP16 Amplicon Sequence

GAGGAAATTC CCGCCTCCAG TTCTGCTGAC GTGGCATCAG CATTGCGGAC GATCTGGCTG TACGGCACCT TCGCTGCATA  
 CAGCGTCCCA TCGTCTAAGC TGGCCTTGAT GACAGCCCCG AAATGTCCAC TGCCGAGGTG CCCACGATTG TATAGAGCGA  
 TCGGCACTCC GTTGGCATTG ATCATCATGA CACGCTCATT TGGAGGAAAC ACTTCGTCAA CAGCTGACTC CACCGCTCCT  
 GGAGGTTGCT TTCTCTTCCG CTTACGGGCA GCAACCCTCT GTTCTGAGAG GACCGCCTCT TGCAGAACAG CCCGCATTGC  
 CTCATAGTCC GGAACCGCTA CGAGAAGCTG TCGGGCAGCT GCTTTCAGCT GCTGAACTTC TTCCAGACCC TCTTCTGAAA  
 GTCGAACGCG CCGGATGCGT TCTGAACGTT TCGCAAGAGA AGCTTGTACT GATACATAAC TAGAATGACT GAAGGCAAGC  
 TGCGAGGCGC CGCTATCTGC TGGATTTCCG GTCGGCATCG CACTCTGTCT TTGAGGGGAT CCCCAGCGCT TTTGTGCCGG  
 AGACTGTATT CCTGAATCCA ATGTAGCGCT CTGACCAGGA AAAAGAGGAT TTTCTTGACC AAGCGACGCA GCGCTCGGCA  
 CCGCCTTTCC TGACAATGCA CTAGAGCTTG CGCCACCGAC GTATCCCTTT GCGGAGGACG CAAGACTGCT CGCTGCGCCA  
 TACTGTTCTT GTGGCTCTTG AAATTCGGTA ACGACCACCC CAGATAGA

ROP18 Amplicon Sequence

AAGTTTGCGG GCACAAAGAC GGCGATCTGA ATTGGTTTTT GAGAAGGCGG ATTCTGGATG CGTCATCGGC AAACGCATCC  
 TGGCGCACAT GCAAGAACAA ATCGGGCAGC CTCAAGCGCT AGAAAATAGT GAACGACTGG ATAGAATTCT GACTGTGCGC  
 GCCTGGCCTC CGGACGTTCC AAAAAAGATTT GTTCTGTGA CTACCGGTGA AACCCGGACG CTGGTGAGAG GTGCACCCCT  
 TGGCTCTGGT GGATTGCGCA CTGTATATGA GGCTACAGAC GTGGAGACGA ATGAAGAGTT GGCTGTTAAG GTTTTCATGT  
 CAGAAAAGGA GCCCACCGAT GAGACTATGC TTGACTTGCA GAGGGAGTCG TCCTGCTACA GGAACCTTAG TCTAGCCAAG  
 ACGGCGAAGG ATGCCCAGGA AAGCTGTAGA TTCATGGTTC CTAGTGATGT TGTGATGTTA GAGGGACAGC CAGCATCCAC  
 AGAGGTCGTG ATTGGTTTGA CGACTCGGTG GGTACCAAAC TATTTTCTTC TCATGATGCG GGCAGAAGCG GACATGAGCA  
 AAGTCATTC ATGGGTATTT GGAGATGCGT CTGTCAATAA AAGTGAATTT GCCTGGTCG TTCGAATGTA CCTATCCAGT  
 CAGGCAATCA AACTAGTGGC CAATGTTCAA GCTCAGGGAA TTGTGCATAC GGATATCAAA CCGGCGAATT

Date: 25 APR 2016

Signature: 

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

