

***Toxoplasma gondii*, Strain RH $\Delta ku80 \Delta rop18$**
Catalog No. NR-49442

Product Description: *Toxoplasma gondii* (*T. gondii*), strain RH $\Delta ku80 \Delta rop18$ was deposited to BEI Resources as a mutant of the virulent Type I strain RH that lacks the *ku80* and *rop18* genes.

Lot^{1,2}: 63721680
Manufacturing Date: 21SEP2015

TEST	SPECIFICATIONS	RESULTS
Cell Morphology	Report results	Refractile and crescent shaped
PCR Assay of Extracted DNA ^{3,4} Ku80 locus ROP5 locus ROP16 locus ROP18 locus	No amplicon ~ 887 base pair amplicon ~ 986 base pair amplicon No amplicon	No amplicon ~ 887 base pair amplicon ~ 986 base pair amplicon No amplicon
Genotypic Analysis ^{3,4} Sequencing of ROP5 locus (~ 780 base pairs) Sequencing of ROP16 locus (885 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	5.0 × 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-49442 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.

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

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AGACTTGCTC AGCACTTCCG TAGGCTGAGG GGCTTCTTCG GACGCCTTAC GCCGAGGTGG CTCTCCGGTC TCGGCCGCCG
GGCGCAAAGA TGGTGGAGAG GGAGACAGAG ACCGCTGCTG GACCCCTTCGT TTCATGGGTT GGAAGCTGGA GATTCGTTCA
TGC GCGACCTT GCTGAAACGT GAARAAGAGC TGATTGGATA CTGTGCGGAA GAAGCGTTGA AAGAACCCTGC AGCGATGGTT
GAGGCTGTCA CGGCAACTGT ATGGCCGCAA AATGCTGAAA CAACCGTGGA TTCRCCTTTTG AGTCAGGGAG AGCGGAAGTT
GAAATTGGTG SAGCCTCTTC GAGTCGGTGA CCGATCTGTC GTATTTTTTAG TAAGGGATGT AGAGCGCCTG GAGKATTTTCG
CTCTGAAGGT CTTCACTATG GGTGCCGAGA ATTCCCAGATC AGAGCTGGAG CGGTTGCATG AAGCGACTTT TGCGGCAGCG
AGGTTGCTTG GGGAGAGTCC AGAGGAGGCA CGGGACAGAC GCAGGCTTTT ACTTCCCTCC GATGYTGTGG CAGTTCAGTC
TCAGCCCCCT TTCGCTCAGC TGAGTCCAGG ACAGRRCGAC TATGCAGTCG CGAACTAYTT GCTTCTCATG CCCGCTGCGT
CGGTGGATCT TGAATTGCTC TTTAGSACAT TGRAYTTCST GTATGTAYTC AGGRGKRSAG AAGRTWTTTT AGCGCKTCAC
ATACTAACGG CACAGCTGAT CCGTCTGGCA GCCAACCTGC AGAGCAAAGG ACTTGTGCAT GGAC

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ROP16 Amplicon Sequence

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CAGAGTCAAT ATATACGCTA TGGAGTTCTC CGCCGCAACG ATTGACACAT CGAAAGCCAT CTCTATCTGG GGTGGTCGTT
ACCGAATTTT AAGAGCCACA AGAACAGTAT GGCGCAGCGA GCAGTCTTGC GTCCTCGCCA AAGGGATACG TCGGTGGCGC
AAGCTCTAGT GCATTGTCAG GAAAGGCGGT GCCGACGCCT GCGTCGCTTG GTCAAGAAAA TCCTCTTTTT CTGGTCAGA
GCGCTACATT GGATTGAGGA ATACAGTCTC CGGCACAAAA GCGTCGGGGA TCCCTCAA GACAGAGTGC GATGCCGACC
GGAAATCCAG CAGATAGCGG CGCCTCGCAG CTTGCCCTTCA GTCATTCTAG TTATGTATCA GTACAAGCTT CTCTTGCGAA
ACGTTTCAGAA CGCATCCGGC GCGTTCGACT TTCAGAAGAG GGTCTGGAAG AAGTTCAGCA GCTGAAAGCA GCTGCCGCAC
AGCTTCTCGT AGCGGTTCCG GACTATGAGG CAATGCGGGC TGTCTGCAA GAGGCGGTCC TCTCAGAACA GAGGGTTGCT
GCCCCGTAAGC GGAAGAGAAA GCAACCTCCA GGAGCGGTGG AGTCAGCTGT TGACGAAGTG TTTCTCCAA ATGAGCGTGT
CATGATGATA AATGCCAACG GAGTGCCGAT CGCTCTATAC AATCGTGGGC ACCTCGGCAG TGGACATTTT GGGGCTGTCA
TCAAGGCCAG CTTAGACGAT GGGACGCTGT ATGCAGCGAA GGTGCCGTAC AGCCAGATCG TCCCGAATGC TGATGCCACG
TCAGCAGAAC TGGAGGCGGG AATTTCTCTCA GCTAGGGCGG AGTTGGTAAA GACAATTCGA CAGGAGTTGG ATGTTGCGGA
TAAGC

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Date: 25 APR 2016

Signature: 

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