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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-51143

## *Toxoplasma gondii*, Strain RH Δ*rop17*

## Catalog No. NR-51143

**Product Description:** *Toxoplasma gondii (T. gondii)*, strain RH Δ*rop17* was deposited to BEI Resources as a mutant of the virulent Type I strain RH created by the deletion of the *rop17* locus.

**Lot[1]: 70012758** **Manufacturing Date: 09MAR2018**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Cell Morphology[2]** | Report results | Refractile, vacuoles present |
| **Genotyping[3]** | | |
| Sequencing of ROP16 locus (~ 860 base pairs) | Consistent with *T. gondii* | Consistent with *T. gondii* (Figure 1) |
| Sequencing of ROP18 locus (~ 750 base pairs) | Consistent with *T. gondii* | Consistent with *T. gondii* (Figure 2) |
| **Confirmation of Genes by PCR Amplification[3-5]** | | |
| ROP16 locus | ~ 990 base pair amplicon | ~ 990 base pair amplicon |
| ROP17 locus | No amplicon | No amplicon |
| ROP17 locus (positive control) | ~ 370 base pair amplicon | ~ 370 base pair amplicon |
| ROP18 locus | ~ 800 base pair amplicon | ~ 800 base pair amplicon |
| **Viable Cell Count by Hemacytometry[3]** | > $10^6$ cells/mL | 5.3 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 | No growth | No growth |
| Trypticase soy broth, 37°C and 26°C, aerobic | No growth | No growth |
| Sabouraud broth, 37°C and 26°C, aerobic | No growth | No growth |
| DMEM with 10% FBS, 37°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, aerobic | No growth | No growth |
| Sheep blood agar, 37°C, anaerobic | No growth | No growth |
| Thioglycollate broth, 37°C, anaerobic | No growth | No growth |
| **Mycoplasma Contamination[2]** | | |
| DNA Detection by PCR | None detected | None detected |

[1]NR-51143 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% $CO_2$ until lysis of the host cell monolayer was reached.
[2]Testing completed on vialed, post-freeze material.
[3]Testing completed on bulk material prior to vialing and freezing.
[4]PCR amplification was performed separately for the three loci ROP16, ROP17 and ROP18. Where appropriate, samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.
[5]Primer sequences and conditions for PCR are available upon request.
[6]Viable cells and signs of infection were seen after 3 days at 37°C in an aerobic atmosphere with 5% $CO_2$ in DMEM supplemented with 10% heat-inactivated fetal bovine serum.
[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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**Figure 1: ROP16 Locus Amplicon Sequence**

ATATATACGC TATGGAGTTC TCCGCCGCAA CGATTGACAC ATCGAAAGCC ATCTCTATCT GGGGTGGTCG TTACCGAATT
TCAAGAGCCA CAAGAACAGT ATGGCGCAGC GAGCAGTCTT GCGTCCTCGC CAAAGGGATA CGTCGGTGGC GCAAGCTCTA
GTGCATTGTC AGGAAAGGCG GTGCCGACGC CTGCGTCGCT TGGTCAAGAA AATCCTCTTT TTCCTGGTCA GAGCGCTACA
TTGGATTCAG GAATACAGTC TCCGGCACAA AAGCGTCGGG GATCCCCTCA AAGACAGAGT GCGATGCCGA CCGGAAATCC
AGCAGATAGC GGCGCCTCGC AGCTTGCCTT CAGTCATTCT AGTTATGTAT CAGTACAAGC TTCTCTTGCG AAACGTTCAG
AACGCATCCG GCGCGTTCGA CTTTCAGAAG AGGGTCTGGA AGAAGTTCAG CAGCTGAAAG CAGCTGCCGC ACAGCTTCTC
GTAGCGGTTC CGGACTATGA GGCAATGCGG GCTGTTCTGC AAGAGGCGGT CCTCTCAGAA CAGAGGGTTG CTGCCCGTAA
GCGGAAGAGA AAGCAACCTC CAGGAGCGGT GGAGTCAGCT GTTGACGAAG TGTTTCCTCC AAATGAGCGT GTCATGATGA
TAAATGCCAA CGGAGTGCCG ATCGCTCTAT ACAATCGTGG GCACCTCGGC AGTGGACATT TCGGGGCTGT CATCAAGGCC
AGCTTAGACG ATGGGACGCT GTATGCAGCG AAGGTGCCGT ACAGCCAGAT CGTCCCGAAT GCTGATGCCA CGTCAGCAGA
ACTGGAGGCG GGAATTTCCT CAGCTAGGGC GGAGTTGGTA AAGACAATTC GACAGGAGTT GGAT

**Figure 2: ROP18 Locus Amplicon Sequence**

TACTTTCCTC AAGGGCGTAA CCGACAGCGA AGTTTGCGGG CACAAAGACG GCGATCTGAA TTGGTTTTTG AGAAGGCGGA
TTCTGGATGC GTCATCGGCA AACGCATCCT GGCGCACATG CAAGAACAAA TCGGGCAGCC TCAAGCGCTA GAAAATAGTG
AACGACTGGA TAGAATTCTG ACTGTCGCCG CCTGGCCTCC GGACGTTCCA AAAAGATTTG TTTCTGTGAC TACCGGTGAA
ACCCGGACGC TGGTGAGAGG TGCACCCCTT GGCTCTGGTG GATTCGCCAC TGTATATGAG GCTACAGACG TGGAGACGAA
TGAAGAGTTG GCTGTTAAGG TTTTCATGTC AGAAAAGGAG CCCACCGATG AGACTATGCT TGACTTGCAG AGGGAGTCGT
CCTGCTACAG GAACTTTAGT CTAGCCAAGA CGGCGAAGGA TGCCCAGGAA AGCTGTAGAT TCATGGTTCC TAGTGATGTT
GTGATGTTAG AGGGACAGCC AGCATCCACA GAGGTCGTGA TTGGTTTGAC GACTCGGTGG GTACCAAACT ATTTTCTTCT
CATGATGCGG GCAGAAGCGG ACATGAGCAA AGTCATTTCA TGGGTATTTG GAGATGCGTC TGTCAATAAA AGTGAATTTG
GCCTGGTCGT TCGAATGTAC CTATCCAGTC AGGCAATCAA ACTAGTGGCC AATGTTCAAG CTCAGGGAAT TGTGCATACG
GATATCAAAC CGGCGAATTT CCTCC

/Heather Couch/
Heather Couch 13 JUN 2018
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