Certificate of Analysis for NR-51144

Toxoplasma gondii, Strain RH Δ*rop17* Δ*rop18*

Catalog No. NR-51144

Product Description: *Toxoplasma gondii (T. gondii)*, strain RH Δ*rop17* Δ*rop18* was deposited to BEI Resources as a mutant of the virulent Type I strain RH created by the deletion of the *rop17* and *rop18* loci.

Lot[1]: 70012023 **Manufacturing Date: 15JAN2018**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[2]	Report results	Refractile and crescent-shaped
Genotyping[3] Sequencing of ROP16 locus (~ 860 base pairs)	Consistent with *T. gondii*	Consistent with *T. gondii* (Figure 1)
Confirmation of Genes by PCR Amplification[3-5] ROP16 locus ROP17 locus ROP17 locus (positive control) ROP18 locus ROP18 locus (positive control)	 ~ 990 base pair amplicon No amplicon ~ 370 base pair amplicon No amplicon ~ 800 base pair amplicon	 ~ 990 base pair amplicon No amplicon ~ 370 base pair amplicon No amplicon ~ 800 base pair amplicon
Viable Cell Count by Hemacytometry[3]	> 10^6 cells/mL	8.9 x 10^6 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 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[2] DNA Detection by PCR	None detected	None detected

[1]NR-51144 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 7 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 7 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 GACAGGAG

/Heather Couch/
Heather Couch 25 JUL 2018
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