Certificate of Analysis for NR-51627

Toxoplasma gondii, Strain RH *Δku80*::DiCre:T2A:CAT

Catalog No. NR-51627

Product Description:

Toxoplasma gondii (T. gondii), strain RH *Δku80*::DiCre:T2A:CAT was deposited to BEI Resources as a transgenic, chloramphenicol-resistant strain, derived from the virulent Type I strain RH. Strain RH *Δku80*::DiCre:T2A:CAT was engineered by transfection of the RH *Δku80* strain with a DiCre_T2A construct, which expresses two dimerizable Cre (DiCre) recombinase subunits from a single promoter using T2A skip peptides. A chloramphenicol acetyltransferase (CAT) selectable marker is located between the two DiCre subunits to prevent the loss of the recombinase.

Lot: 70026739[1] **Manufacturing Date: 24JUN2019**

TEST	SPECIFICATIONS	RESULTS
Cell Morphology[2]	Report results	Crescent-shaped and refractile
Genotypic Analysis[3]		
Sequencing of 850 locus (~ 700 base pairs)	≥ 99% sequence identity to *T. gondii*, strain RH KT850 (GenBank: GU249505)	100% sequence identity to *T. gondii*, strain RH KT850 (GenBank: GU249505) (Figure 1)
Sequencing of Cre recombinase (~ 250 base pairs)	Consistent with Cre recombinase	Consistent with Cre recombinase (Figure 2)
PCR Assay of Extracted DNA[3,4]		
850 locus	~ 770 base pair amplicon	~ 770 base pair amplicon
Cre recombinase	~ 260 base pair amplicon	~ 260 base pair amplicon
ku80 gene	No amplicon detected	No amplicon detected
Viable Cell Count by Hemacytometry[3]	> 10^6 cells per mL	5.6 × 10^7 cells per mL
Viability (post-freeze)[2,5]	Growth	Growth
Sterility (21-day incubation)[2]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[6]	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-51627 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: Dulbecco's Minimal Essential Medium 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% 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]Primer sequences and PCR conditions are available upon request.
[5]Viable cells were seen after 3 days at 27°C in an aerobic atmosphere in DMEM supplemented with 10% heat-inactivated fetal bovine serum.
[6]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: 850 Locus Amplicon Sequence

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CCTTCTCCAG CATCTGGGGG GAAACCCGTA GAGGCGCCTT TACATTCCAC ACCTGCAACG GGGAGTCCTT CAGAGACTGT CCGTGTACCA
GCAGACACAC AAAGTCCGTG CTGCGGCGCA AAGGGCTCGC TTCCAAAGGA AACATCCCCG AAAAGCGACA TCGGAACACA CGTTCCCGGA
AAACGTTCGC CGCCATCACC GTCCCCACTG CAAGGCCCGC GTCTTGCTTC ATTTTTCGTG GGCTCTGAGG GCGGGCCAGA GGGTGACGTC
GTCATAATCC ACTCAGGTGG GCCTTCTTCG TCGTCAGGCA GCAGCGGCTT GGGCGACAGC TCAGTTTCAC CGTCAGATGG TGACTCACCG
ACGCCAAGCG CTCCTGCACG AGGCATGCAG CAAGAGGGAC CTGTGGGGGG CAGCAGCTCA CCGGAGCAAG GAGGGAGGCA GGCACAGGCA
CCCATTAGTG CCACTACTGC TTCCTCCCCA CAAACAGGGA TGCCTCCAGG GAAAGCAGCG CCGACACCCA GAAGCGATGG CAAGCCGTCG
CCAGGGCGAA CGGGCGTCGA TAAGGTCGGA GGGCCTTCTC GTGGAAGAGG GTCGCCTTCG CGTAGCCCCG GTAGCCGGTT GAGCCTTTTA
AAGACCCCAC GGGGACCAGT TGCCTCACCG ACAGGGGGGC TACGAGGAAG GGGTGGCAGC CATGCAGCAT CG
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Figure 2: Cre Recombinase Amplicon Sequence

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AGTGCGTTCA AAGGCCAGGG CCTGCTTGGC TCTCTCCCCA GCATCCACAT TCTCCTTTCT GATTCTCCTC ATCACCAGGG ACACAGCATT
GGAGTCAGAA GGGCGAGGCA GGCCAGATCT CCTGTGCAGC ATGTTGAGCT GGCCCAGGTG CTGTTGGATG GTCTTCACAG CCAGGCCTCT
GGCTTGCAGG TACAGGAGGT AGTCCCTCAC ATCCTCAGGT TCAGCAGGGA ACCATTTCCT GTTGGATCC
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/Heather Couch/
Heather Couch 15 OCT 2019
Program Manager or designee, ATCC Federal Solutions

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