Certificate of Analysis for NR-10158

Toxoplasma gondii, Clone CL16

Catalog No. NR-10158

Product Description: *Toxoplasma gondii*, clone CL16 is a recombinant F1 clone selected from progeny of two parallel genetic crosses between a Type II parental strain [ME49 (clone B7)] and a Type III parental strain (CTG ARA-SYN).

Lot[1]: 58270246 **Manufacturing Date: 13AUG2008**

TEST	SPECIFICATIONS	RESULTS
Genotyping[2]		
850 locus (*Sfa* NI digestion)[3]	Consistent with parental Type III strain	Consistent with parental Type III strain
SAG1 locus[4]	Consistent with parental Type III strain	Consistent with parental Type III strain
Drug susceptibility[5]		
Sinefungin	Resistant	Resistant
Ara-A	Susceptible	Susceptible
Viable Cell Count by Hemacytometry (pre-freeze)	> 10^6 cells/mL	1.5 x 10^7 cells/mL
Viability (post-freeze)[6]	Growth	Growth
Sterility (21-day incubation)		
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
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
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	No growth
Mycoplasma Contamination		
DNA Detection by PCR	None detected	None detected

[1]NR-10158 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: adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated in 95% air, 5% CO_2 for 8 days at 37°C, until lysis of the host cell monolayer was reached.

[2]PCR amplification was performed separately for the two loci 850 and SAG1. Where appropriate, samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.

[3]Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the *Toxoplasma* Genome Map website (Toxoplasma Genome Map). [4]Primer sequences and conditions for PCR are available upon request.

[5]Sinefungin was used at a concentration of 2.7 x 10^{-7} M and ara-A was used at a concentration of 1.3 x 10^{-4} M, as described (Sibley, L. D., et al. "Generation of a Restriction Fragment Length Polymorphism Linkage Map for *Toxoplasma gondii*." Genetics 132 (1992): 1003-1015. PubMed: 1360931.)

[6]Viable cells were seen after 2 days under cultivation conditions at 37°C.

[7]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 15 OCT 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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