b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-10262

Toxoplasma gondii, Clone c285-31

Catalog No. NR-10262

Product Description: *Toxoplasma gondii*, c285-31 is a virulent recombinant F1 clone selected from progeny of two parallel genetic crosses between a highly virulent Type I parental strain, GT1-FUDR3.3 and a less virulent Type III parental strain, CTG.11 ARA-SNF.

Lot[1]: 59095942 **Manufacturing Date: 17MAR2010**

TEST	SPECIFICATIONS	RESULTS
Genotyping[2,3]		
AK16 locus (*Hinf*I digestion)	Consistent with parental Type III strain	Consistent with parental Type III strain
L358 locus (*Hae*III digestion)	Consistent with parental Type III strain	Consistent with parental Type III strain
Drug susceptibility[4]		
Sinefungin (SNF)	Resistant	Resistant
Adenine arabinose (Ara-A)	Resistant	Resistant
Viable Cell Count by Hemacytometry (pre-freeze)	> 10^6 cells/mL	2.9 x 10^7 cells/mL
Viability (post-freeze)[5]	Growth	Growth
Sterility (21-day incubation)		
Harpo's HTYE broth[6], 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
Brain heart infusion, 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
Mycoplasma Contamination		
DNA Detection by PCR	None detected	None detected

[1]NR-10262 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 5 days at 37°C, until lysis of the host cell monolayer was reached.

[2]PCR amplification was performed separately for the two loci AK16 and L358. 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]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).

[5]Viable cells and signs of infection were seen after 15 days under cultivation conditions at 37°C.

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

Date: 24 JUN 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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