

***Toxoplasma gondii*, Clone c295-3**

Catalog No. NR-10254

Product Description: *Toxoplasma gondii*, c295-3 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.

Lot¹: 58840399

Manufacturing Date: 23OCT2009

TEST	SPECIFICATIONS	RESULTS
Genotyping^{2,3} AK16 locus (<i>Hinf</i> I digestion) L358 locus (<i>Hae</i> III digestion)	Consistent with parental Type III strain Consistent with parental Type III strain	Consistent with parental Type III strain Consistent with parental Type III strain
Drug susceptibility⁴ Sinefungin (SNF) Adenine arabinose (Ara-A)	Resistant Resistant	Resistant Resistant
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	2 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 Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	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 DNA Detection by PCR	None detected	None detected

¹NR-10254 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% CO₂ for 4 days at 37°C, until lysis of the host cell monolayer was reached.

²PCR 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.

³Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the *Toxoplasma* Genome Map website ([Toxoplasma Genome Map](#)).

⁴Sinefungin 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).

⁵Viable cells and signs of infection were seen after 6 days under cultivation conditions at 37°C.

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

Date: 16 MAR 2010

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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