

Certificate of Analysis for NR-10164

Toxoplasma gondii, Clone S25

Catalog No. NR-10164

Product Description: Toxoplasma gondii, clone S25 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).

Lot¹: 58319478 Manufacturing Date: 06OCT2008

TEST	SPECIFICATIONS	RESULTS
Genotyping ²		
850 locus (Sfa NI digestion) ³	Consistent with parental Type II strain	Consistent with parental Type II strain
SAG1 locus ⁴	Consistent with parental Type II strain	Consistent with parental Type II strain
Drug susceptibility ⁵		
Sinefungin	Resistant	Resistant
Ara-A	Susceptible	Susceptible
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	2.0 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 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	No growth No growth No growth No growth No growth 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

¹NR-10164 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 7 days at 37°C, until lysis of the host cell monolayer was reached.

Date: 16 OCT 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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²PCR 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.

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

⁵Sinefungin 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.*" <u>Genetics</u> 132 (1992): 1003-1015. PubMed: 1360931.)

⁶Viable cells and signs of infection were seen after 4 days under cultivation conditions at 37°C.

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