

Certificate of Analysis for NR-10170

Toxoplasma gondii, Strain CL15

Catalog No. NR-10170

Product Description: *Toxoplasma gondii* (*T. gondii*), strain CL15 is a recombinant F1 clone selected from progeny of two parallel genetic crosses between a Type II parental strain [ME49 (clone B7); BEI Resources NR-10150] and a Type III parental strain (CTG ARA-SNF; BEI Resources NR-10151).

Lot¹: 58681297 Manufacturing Date: 29JUN2009

TEST	SPECIFICATIONS	RESULTS
Genotyping ² 850 locus (Sfa NI digestion) ³ SAG1 locus ⁴	Consistent with parental Type II strain Consistent with parental Type III strain	Consistent with parental Type II strain Consistent with parental Type III strain
Drug Susceptibility ⁵ Sinefungin Adenine arabinoside (ara-A)	Susceptible Susceptible	Susceptible Susceptible
Viable Cell Count by Hemacytometry (pre-freeze)	> 10 ⁶ cells/mL	7.3 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 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-10170 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 for 6 days at 37°C in 95% air, 5% CO₂, until lysis of the host cell monolayer was reached.

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Date: 12 MAR 2015 Signature:

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 (http://toxomap.wustl.edu/Toxo Genetic Map Table.html).

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 (see, 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.).

⁶Incubated for 23 days under cultivation conditions

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