## Toxoplasma gondii, Clone C11 AF

## Catalog No. NR-10245

Product Description: Toxoplasma gondii, C11 AF is a virulent recombinant F1 clone selected from progeny of two parallel genetic crosses between a highly virulent Type I parental strain,GT1FUDR3.3 and a less virulent Type III parental strain, CTG. 11 ARA-SNF.
Lot ${ }^{1}$ : 58638995
Manufacturing Date: 10JUL2009

| TEST | SPECIFICATIONS | RESULTS |
| :---: | :---: | :---: |
| $\begin{aligned} & \text { Genotyping }{ }^{2,3} \\ & \text { AK16 locus (Hinf digestion) } \\ & \text { L358 locus (Haelll digestion) } \end{aligned}$ | Consistent with parental Type I strain Consistent with parental Type III strain | Consistent with parental Type I strain Consistent with parental Type III strain |
| Drug susceptibility ${ }^{4}$ Sinefungin (SNF) Adenine arabinose (Ara-A) | Resistant Resistant | Resistant Resistant |
| Viable Cell Count by Hemacytometry (pre-freeze) | $>10^{6}$ cells $/ \mathrm{mL}$ | $5.2 \times 10^{7}$ cells $/ \mathrm{mL}$ |
| Viability (post-freeze) ${ }^{5}$ | Growth | Growth |
| Sterility (21-day incubation) <br> Harpo's HTYE broth ${ }^{6}, 37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic Trypticase soy broth, $37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic Sabouraud broth, $37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic Sheep blood agar, $37^{\circ} \mathrm{C}$, aerobic Sheep blood agar, $37^{\circ} \mathrm{C}$, anaerobic Thioglycollate broth, $37^{\circ} \mathrm{C}$, anaerobic DMEM with $10 \% \mathrm{FBS}, 37^{\circ} \mathrm{C}$ and $5 \% \mathrm{CO}_{2}$ | No growth <br> No growth <br> No growth <br> No growth <br> No growth <br> No growth <br> No growth | No growth <br> No growth <br> No growth <br> No growth <br> No growth <br> No growth <br> No growth |
| Mycoplasma Contamination DNA Detection by PCR | None detected | None detected |

${ }^{1}$ NR-10245 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC ${ }^{\text {® }}$ CRL-1634 ${ }^{\text {TM }}$ ) 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, $5 \%$ $\mathrm{CO}_{2}$ for 4 days at $37^{\circ} \mathrm{C}$, 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 $2.7 \times 10^{-7} \mathrm{M}$ and ara-A was used at a concentration of $1.3 \times 10^{-4} \mathrm{M}$, 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 9 days under cultivation conditions at $37^{\circ} \mathrm{C}$.
${ }^{6}$ Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.
Date:27 OCT 2009

## Signature: Signature on File

## Title: Technical Manager, BEI Authentication or designee

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