## Certificate of Analysis for NR-49199

SUPPORTING INFECTIOUS DISEASE RESEARCH

## Toxoplasma gondii, Strain SF61

## Catalog No. NR-49199

Product Description: Toxoplasma gondii (T. gondii), strain SF61 is a recombinant F1 clone selected from progeny of a genetic cross between a sinfungin-resistant line of the highly virulent Type I GT-1 strain (GT1-SNFR) and a 5-fluoro-2'-deoxyuridine-resistant line of the non-virulent Type II ME49 strain (ME49 FUDR ${ }^{\mathrm{R}}$ ).

Lot ${ }^{1,2}$ : 64312880
Manufacturing Date: 24MAY2016

| TEST | SPECIFICATIONS | RESULTS |
| :---: | :---: | :---: |
| Cell Morphology | Report results | Refractile and oval-shaped |
| PCR Assay of Extracted DNA ${ }^{3,4}$ AK56 locus | ~ 520 base pair amplicon | ~ 520 base pair amplicon |
| Genotypic Analysis ${ }^{3,4}$ <br> Sequencing of AK56 locus (~ 510 base pairs) <br> AK56 locus (Mfel digestion) | Consistent with T. gondii <br> Consistent with parental Type II strain | Consistent with T. gondii (Figure 1) Consistent with parental Type II strain |
| Viable Cell Count by Hemacytometry (pre-freeze) | > $10^{6}$ cells $/ \mathrm{mL}$ | $1.7 \times 10^{7} \mathrm{cells} / \mathrm{mL}$ |
| Viability (post-freeze) ${ }^{5}$ | Viable parasites | Viable parasites |
| Sterility (21-day incubation) <br> Harpo's HTYE broth ${ }^{6}, 37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic Tryptic Soy broth, $37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic Sabouraud Dextrose broth, $37^{\circ} \mathrm{C}$ and $26^{\circ} \mathrm{C}$, aerobic DMEM with $10 \% \mathrm{FBS}, 37^{\circ} \mathrm{C}$, aerobic Sheep Blood agar, $37^{\circ} \mathrm{C}$, aerobic Sheep Blood agar, $37^{\circ} \mathrm{C}$, anaerobic Thioglycollate broth, $37^{\circ} \mathrm{C}$, anaerobic | 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-49199 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC ${ }^{\circledR}$ CRL-1634 ${ }^{\text {TM }}$ ) with cell cultivation medium for parasites (ATCC ${ }^{\circledR}$ medium 2222: adjusted to contain $10 \%$ heat-inactivated fetal bovine serum). The culture was propagated for 4 days at $37^{\circ} \mathrm{C}$ in an aerobic atmosphere with $5 \% \mathrm{CO}_{2}$ until lysis of the host cell monolayer was reached.
${ }^{2}$ Quality control testing completed on post-freeze material unless specified as pre-freeze.
${ }^{3}$ PCR amplification of the AK56 locus was performed. Samples were subjected to restriction enzyme digestion typing by agarose gel electrophoresis.
${ }^{4}$ Primer sequences, annealing temperatures, and conditions for restriction enzyme digestion may be obtained at the Toxoplasma Genome Map website (Toxoplasma Genome Map).
${ }^{5}$ Viable cells and signs of infection were seen after 1 day at $37^{\circ} \mathrm{C}$ in an aerobic atmosphere with $5 \% \mathrm{CO}_{2}$.
${ }^{6}$ Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.
Figure 1: AK56 (Chromosome II) Amplicon Sequence
TTGTCCTTTT CCCCACTGCT AAATCTCGTT TCAACGTATC CTTTTAGCCG GCTCGTTTTT TGATCACCGT ATGAAAATCG ACGAACTCTC AGCAAGAATT TTCGAGCACT ACTGCGTCGC AGCAGCCTAG TGGGGTGGAC ACGCATGTGC AGGACGGACA GAAACTGCAA GCTTGTTCCG CAGGCTAAAA CTC


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