

Certificate of Analysis for NR-20738

Toxoplasma gondii, Strain VAND

Catalog No. NR-20738

Product Description: *Toxoplasma gondii* (*T. gondii*), strain VAND was isolated from a human in French Guyana in 1997. Strain VAND was deposited as a prototype strain for the type X haplogroup and is a reference strain for the *Toxoplasma gondii* Genome Project at the J. Craig Ventor Institute's Genomic Sequencing Center for Infectious Diseases (GSCID).

Lot¹: 59907710 Manufacturing Date: 08APR2011

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| Genotyping Sequencing of uracil phosphoribosyltransferase (UPRT) intron 1 (~ 470 bp) | Consistent with <i>T. gondii,</i> haplotype X | Consistent with <i>T. gondii,</i> haplotype X ² (Figure 1) |
| Functional Activity by PCR Amplification ³ UPRT intron 1 | ~ 560 bp amplicon | ~ 560 bp amplicon |
| Viable Cell Count by Hemacytometry (pre-freeze) | > 10 ⁶ cells/mL | 8 x 10 ⁶ cells/mL |
| Viability (post-freeze) ⁴ | Growth | Growth |
| Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Brain heart infusion, 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 |
| Mycoplasma Contamination DNA Detection by PCR | None detected | None detected |

¹NR-20738 was produced by cultivation of the deposited material in human foreskin fibroblast cells (ATCC[®] CRL-1634™) with cell cultivation medium for parasites (<u>ATCC medium 2222:</u> adjusted to contain 10% heat-inactivated fetal bovine serum). The culture was propagated in 95% air, 5% CO₂ for 3 days at 37°C, until lysis of the host cell monolayer was reached.

Figure 1: Toxoplasma gondii, Strain VAND - UPRT Intron 1 Sequence

| GTAATCCTTC | AACCGAAGTT | TGCTTTCCGT | GACTCTGCCT | CTTGGTTATA | CTGCGTGGCC | TTCCCGTCCT | GCCGCCCCT | |
|------------|------------|------------|------------|------------|------------|------------|------------|--|
| TTCCTCCGCT | TGCTGTTTAA | ATGCTCGTCC | TCGTTTTCCT | TCCTGCCGCA | TCCCCGTATA | TTTTAAGGAG | AGGGAAACAG | |
| GCGTGAGTTG | GACGGAATGA | AAGTTCTCGG | CCTGTACGCC | GGTTGTCGCG | GTCGTTTGCA | GATTGCTTTT | TTCTTCGAAT | |
| CGGTGCTGTA | ACCCTCGAAG | AAGAACGACG | CTGCAAACGA | CTTCTCGAAC | TCTCAGTCGT | GTACTTTACG | TGCTTCCTTT | |
| CAGGGACCTC | CCCCGCGTT | ACTCATTTGT | ATTCACAGCT | ACGAAGTGTC | TTGCAAGGTG | GATTTCTGCC | AGGCTCCATG | |
| TCTCACTCGG | тесеттттсе | GAAAAGTTCA | ТТСТСААССТ | TCCCCTTGCG | ТСТСАТСАСТ | TTATCAG | | |

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²Haplotype identification is based on a sequence alignment of NR-20738, lot 59907710, to a reference sequence provided by the depositor. See figure 1 for NR-20738 sequence.

³Primer sequences and conditions for PCR are available upon request.

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

⁵Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Date: 23 SEP 2011 Signature: Value one

Title: Technical Manager, BEI Authentication or designee

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