

Certificate of Analysis for MRA-1173

Plasmodium falciparum, Strain SenP051.02

Catalog No. MRA-1173

Product Description: Plasmodium falciparum (P. falciparum), strain SenP051.02 (also referred to as P51.02) was isolated in 2002 from the venous blood of a patient with mild malaria in Pikine, Senegal, and adapted to culture at the Harvard School of Public Health, Boston, Massachusetts, USA. Strain SenP051.02 was deposited as genotype CACTGCGGTTTATCAATTAGCCTG (24-SNP bar code).

Lot¹: 61535349 Manufacturing Date: 20MAR2013

| TEST | SPECIFICATIONS | RESULTS | | |
|--|--|---|--|--|
| Identification by Giemsa Stain Microscopy ² | Blood-stage parasites present | 31.1 ± 1.4 nM 5.6 ± 0.4 nM 78.3 ± 2.5 nM 499.8 ± 27.0 nM 16360 ± 1518.2 nM 338900 ± 57219 nM | | |
| Antimalarial Susceptibility Profile (in vitro) Half-maximal Inhibitory Concentration (IC50) by SYBR green I® drug sensitivity assay³ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine | Report results | | | |
| Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 base pairs) MSP2 PCR amplicon analysis ⁴ | Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon | Consistent with <i>P. falciparum</i> (Figure 1) ~ 900 base pair amplicon | | |
| Level of Parasitemia Pre-freeze ⁵ Ring-stage parasitemia Total parasitemia Post-freeze ⁶ Ring-stage parasitemia Total parasitemia | Report results ≥ 2% Report results ≥ 1% | 4.11% 5.48% 1.43% 2.29% | | |
| Viability (post-freeze) ⁷ | Growth in infected red blood cells | Growth in infected red blood cells | | |
| Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°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 1MRA-1173 was produced by cultivation of the deposited mate | None detected | None detected | | |

MRA-1173 was produced by cultivation of the deposited material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 g/L D-glucose, 0.005 μg/mL hypoxanthine and 2.5 μg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia daily for 49 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

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²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 4 days.



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Figure 1: MRA-1173 MSP2 Sequence

| AAGGTAATTA | AAACATTGTC | TATTATAAAT | TTCTTTATTT | TTGTTACCTT | TAATATTAAA | AATGAAAGTA | AATATAGCAA |
|------------|------------|------------|------------|------------|------------|------------|------------|
| CACATTCATA | AACAATGCTT | ATAATATGAG | TATAAGGAGA | AGTATGGCAA | ATGAAGGTTC | TAATACTAAT | AGTGTAGGTG |
| CAAATGCTCC | AAAAGCTGAT | ACTATTGCTA | GTGGAAGTCA | AAGTAGTACA | AATAGTGCAA | GTACTAGTAC | TACTAATAAT |
| AGAGAATCAC | AAACTACTAC | TCCTACCGCT | GCTGATACCC | CTACTGCTAC | AGAAAGTAAT | TCACCTTCAC | CACCCATCGC |
| TACTACAGAA | AGTAATTCAC | CTTCACCACC | CATCACTACT | ACAGAAAGTA | ATTCACCTTC | ACCACCCATC | ACTACTACAG |
| AAAGTTCAAG | TTCTGGCAAT | GCACCAAATA | AAACAGACGG | TAAAGGAGAA | GAGAGTGAAA | AACAAAATGA | ATTAAATGAA |
| TCAACTGAAG | AAGGACCCAA | AGCTCCACAA | GAACCTCAAA | CGGCAGAAAA | TGAAAATCCT | GCTGCACCAG | AGAATAAAGG |
| TACAGGACAA | CATGGACATA | TGCATGGTTC | TAGAAATAAT | CATCCACAAA | ATACTTCTGA | TAGTCAAAAA | GAATGTACCG |
| ATGGTAACAA | AGAAAACTGT | GGAGCAGCAA | CATCCCTCTT | AAATAACTCT | AGTAATATTG | CTTCAATAAA | TAAATTTGTT |
| GTTTTAATTT | CAGCAACACT | TGTTTTATCT | TTTGCCATA | | | | |

Date: 25 OCT 2017 Signature:

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³A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ values of an active (> 70% ring stage) parasite culture in the presence of each antimalarial drug [Hartwig, C. L., et al. "XI: I. SYBR Green I®-Based Parasite Growth Inhibition Assay for Measurement of Antimalarial Drug Susceptibility in Plasmodium falciparum." In Methods in Malaria Research Sixth Edition. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. Available at: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx].

⁴Primer sequences and conditions for PCR are available upon request.

⁵Pre-freeze parasitemia was determined after 49 days post infection by microscopic counts of Giemsa-stained blood smears.

⁶Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.

⁸Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.