Certificate of Analysis for MRA-1177

Plasmodium falciparum, Strain SenP019.04

Catalog No. MRA-1177

Product Description: *Plasmodium falciparum (P. falciparum)*, strain SenP019.04 (also referred to as P19.04) was isolated in 2004 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 SenP019.04 was deposited as genotype CACTCCAGATTGCAACTTAGCTTG (24-SNP bar code).

Lot[1]: 61535345 **Manufacturing Date: 21MAR2013**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	6.9 ± 0.3 nM
Artemisinin	Report results	14.5 ± 0.7 nM
Quinine	Report results	77.0 ± 8.9 nM
Cycloguanil	Report results	526.0 ± 36.4 nM
Pyrimethamine	Report results	23260 ± 3765 nM
Sulfadoxine	Report results	336500 ± 54472 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 560 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]		
Ring-stage parasitemia	Report results	3.35%
Total parasitemia	≥ 2%	4.81%
Post-freeze[6]		
Ring-stage parasitemia	Report results	1.14%
Total parasitemia	≥ 1%	1.99%
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	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Sheep Blood agar, 37°C, aerobic	No growth	No growth
Sheep Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination		
DNA Detection by PCR	None detected	None detected

[1]MRA-1177 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 34 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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[2]Blood-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.
[3]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} 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].
[4]Primer sequences and conditions for PCR are available upon request.
[5]Pre-freeze parasitemia was determined after 34 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1177 MSP2 Sequence

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ATTTATTTAT TGAAGCAATA TTACTAGAGT TATTTAAGAG GGATGTTGCT GCTCCACAGT TTTCTTTGTT ACCATCGGTA
CATTCTTTTT GACTATCAGA AGTATTTTGT GGATGATTAT TTCTAGAACC ATGCATATGT CCATGTTGKC CTGTACCTTT
ATTCTCTGGT GCAGATTGTA ATTCGGGGGA TTCAGTTTGT TCGGCTGTTG GAGCAGAATT TTCAGCTTGT TCAGGTTGTG
CAGTAGGACT TTTAGTGTCT GCATCTTGAG TGGGTGGAAC ATTTGATTTA GTTTGAGAGT CTTGTTGAAC ATTTGAGTTA
TTTTGAGTTT CTGTATTTGC TTGATTTGGT TCTTGAACTC TTCCATTACC TTTTGGATTT GTTTTGGCAT TATTATGATT
TGGATTTTCT GAAGAGGTAC TGGTAGATGC TTCTGCATCA TTAGTAGTTG TGGTAGTTGT GGTAGTAGCG GGAGTACTTG
AACTTCCCTC AGCACCACCA CTACCACTAG CACCACCACT ACCACTAGCA CCACCACTAC CACTAGCACC ACCACTACCA
CTA
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Date: 26 OCT 2017 **Signature:**

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