Certificate of Analysis for MRA-1176

Plasmodium falciparum, Strain SenP011.02

Catalog No. MRA-1176

Product Description: *Plasmodium falciparum (P. falciparum)*, strain SenP011.02 (also referred to as P11.02) 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 SenP011.02 was deposited as genotype TATTCCGAACCGTACCCTCGATTG (24-SNP bar code).

Lot[1]: 61535346 **Manufacturing Date: 14MAR2013**

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	6.7 ± 0.5 nM
Artemisinin	Report results	6.5 ± 0.3 nM
Quinine	Report results	36.1 ± 3.3 nM
Cycloguanil	Report results	6.3 ± 0.4 nM
Pyrimethamine	Report results	29.8 ± 2.1 nM
Sulfadoxine	Report results	478900 ± 33107.6 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 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	3.75%
Total parasitemia	≥ 2%	4.79%
Post-freeze[6]		
Ring-stage parasitemia	Report results	0.30%
Total parasitemia	≥ 1%	1.21%
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-1176 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 27 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 27 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-1176 MSP2 Sequence

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TATGAAGGTA ATTAAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA
GCAACACATT CATAAACAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAAATGAAG GTTCTAATAC TAATAGGGTA
GATGCAAATG CTCCAAAAGC TGATACTAAT GCTAGTGGAA GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA
TAATGGAGAA TCACAAACTA CTACTCCTAC CGCTGCTGAT ACCCCTACTG CTACAAAAAG TAATTCACCT TCATCACCCA
TCACTACTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTAATTCAC CTTCACCACC CATCACTACT
ACAGAAAGTT CAAGTTCTGG CAATGCACCA AATAAAACAG ACGGTAAAGG AGAAGAGAGT GAAAAACAAA ATGAATTAAA
TGAATCAACT GAAGAAGGAC CCAAAGCTCC ACAAGAACCT CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA
AAGGTACAGG ACAACATGGA CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT
ACCGATGGTA ACAAAGAAAA CTGTGGAGCA GCAACATCCC TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT
TGTTGTTTTA ATTTCAGCAA CACTTGTTTT ATCTTTTGCC ATA
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Date: 25 OCT 2017 **Signature:**

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