b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1171

Plasmodium falciparum, Strain SenP026.04

Catalog No. MRA-1171

Product Description: *Plasmodium falciparum (P. falciparum)*, strain SenP026.04 (also referred to as P26.04) 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 SenP026.04 was deposited as genotype TACTGGAAACTGCAACCAAACTTG (24-SNP bar code).

Lot[1]: 61535355 **Manufacturing Date: 15FEB2013**

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	29.5 ± 1.4 nM
Artemisinin	Report results	4.9 ± 0.1 nM
Quinine	Report results	22.0 ± 2.5 nM
Cycloguanil	Report results	390.2 ± 27.0 nM
Pyrimethamine	Report results	16460 ± 1518.2 nM
Sulfadoxine	Report results	495900 ± 57218.8 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 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	3.39%
Total parasitemia	≥ 2%	4.06%
Post-freeze[6]		
Ring-stage parasitemia	Report results	0.73%
Total parasitemia	≥ 1%	2.66%
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-1171 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 16 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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[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 16 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-1171 MSP2 Sequence

```
TTAAAAATGA AAGTAAATAT AGCAACACAT TCATAAACAA TGCTTATAAT ATGAGTATAA GGAGAAGTAT GACAGAAAGT
AAGACTCCTA CTGGTGCTGG TGCTGGTGCT AGTGGTAATG CTGGTGCTGG TGCTGGTGCT GGTGCTAGTG GTAGTGCTGG
TTCTGGTGAT GGTAATGGTG CTAATCCTGG TGCAGATGCT GAGAGAAGTC CAAGTACTCC CGCTACTCCC GCTACTACCA
CAACTACCAC AACTACTAAT GATGCAGAAG CATCTACCAG TACCTCTTCA GAAAATCCAA ATCATAATAA AGCCGAAACA
AATCCAAAAG GTAAAGGAGA AGTTCAAAAA CCAAATCAAG CAAATAAAGA AACTCAAAAT AACTCAAATG TTCAACAAGA
CTCTCAAACT AAATCAAATG TTCCACCCAC TCAAGATGCA GACACTAAAA GTCCTACTGC ACAACCTGAA CAAGCTGAAA
ATTCTGCTCC AACAGCCGAA CAAACTGAAT CCCCCGAATT ACAATCTGCA CCAGAGAATA AAGGTACAGG ACAACATGGA
CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT ACCGATGGTA ACAAAGAAAA
CTGTGGAGCA GCACCATCCC TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTGTTTTA ATTTCAGCAA
CACTTGTTTT ATCTTTTGCC ATA
```

Date: 25 OCT 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898