b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-176

Plasmodium falciparum, Strain V1/S

Catalog No. MRA-176

Product Description:

Plasmodium falciparum (P. falciparum), strain V1/S is an *in vitro* culture-adapted clone of the V1 strain originating in Vietnam, which shows resistance to chloroquine and quinine. MRA-176 was produced by cultivation of BEI seed material (lot 58985215) 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 grams per liter D-glucose, 0.005 µg per mL hypoxanthine and 2.5 µg per 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 for 14 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.

Lot: 70046150 **Manufacturing Date: 29JUL2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	61.4 ± 2.8 nM
Artemisinin	Report results	5.2 ± 0.5 nM
Quinine	Report results	137.9 ± 12.7 nM
Cycloguanil	Report results	227 ± 15.7 nM
Pyrimethamine	Report results	26610 ± 2454 nM
Sulfadoxine	Report results	310200 ± 35792 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (14 days post-infection)[3]		
Ring-stage parasitemia	Report results	3.78%
Total parasitemia	≥ 2%	6.39%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	5.43%
Total parasitemia	≥ 1%	6.39%
Viability (post-freeze; 4 days post-infection)[1]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[4]	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud 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[1]		
DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material

BEI Resources
www.beiresources.org

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

[2]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.]
[3]Testing completed on bulk material prior to vialing and freezing
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-176 MSP2 Sequence

```
TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAATGCTT
ATAATATGAG TATAAGGAGA AGTATGGAAG AAAGTAATCC TTCTACTGGT GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT
GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTGG TAGTGGTAGT GCTGGTGGTA GTGGTAGTGC
TGGTGGTAGT GGTAGTGCTG GTTCTGGTGA TGGTAATGGT GCTAATCCTG GTGCAGATGC TGAGAGAAGT CCAAGTACTC
CCGCTACTAC CACAACTACC ACAACTACTA ATGATGCAGA AGCATCTACC AGTACCTCTT CAGAAAATCC AAATCATAAT
AATGCCGAAA CAAATCCAAA AGGTAAAGGA GAAGTTCAAA AACCAAATCA AGCAAATAAA GAAACTCAAA ATAACTCAAA
TGTTCAACAA GACTCTCAAA CTAAATCAAA TGTTCCACCC ACTCAAGATG CAGACACTAA AAGTCCTACT GCACAACCTG
AACAAGCTGA AAATTCTGCT CCAACAGCCG AACAAACTGA ATCCCCCGAA TTACAATCTG CACCAGAGAA TAAAGGTACA
GGACAACATG GACATATGCA TGGTTCTAGA AATAATCATC CACAAAATAC TTCTGATAGT CAAAAAGAAT GTACCGATGG
TAACAAAGAA AACTGTGGAA CAGCAACATC CCTCTTAAAT AACTCTAGTA ATATT
```

/Heather Couch/
Heather Couch 13 JAN 2022
Program Manager or designee, ATCC Federal Solutions

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.