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.

Lot[1]: 61875546 **Manufacturing Date: 19JUL2013**

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 (IC50) by SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	54.4 ± 3.8 nM
Artemisinin	Report results	7.8 ± 0.5 nM
Quinine	Report results	166.1 ± 11.5 nM
Cycloguanil	Report results	276 ± 64.1 nM
Pyrimethamine	Report results	36290 ± 3347.2 nM
Sulfadoxine	Report results	556900 ± 77183.6 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 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	2.20%
Total parasitemia	≥ 2%	3.18%
Post-freeze[6]		
Ring-stage parasitemia	Report results	1.14%
Total parasitemia	≥ 1%	1.70%
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-176 was produced by cultivation of MR-MRA-176 lot 58928215 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 22 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.

[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.

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

[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 22 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-176 MSP2 Sequence

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TTATTTTTGT TACCTTTAAT ATTAAAAATG AAAGTAAATA TAGCAACACA TTCATAAACA ATGCTTATAA TATGAGTATA
AGGAGAAGTA TGGAAGAAAG TAATCCTTCT ACTGGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTGG
TAGTGGTAGT GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA
GTGCTGGTTC TGGTGATGGT AATGGTGCTA ATCCTGGTGC AGATGCTGAG AGAAGTCCAA GTACTCCCGC TACTACCACA
ACTACCACAA CTACTAATGA TGCAGAAGCA TCTACCAGTA CCTCTTCAGA AAATCCAAAT CATAATAATG CCGAAACAAA
TCCAAAAGGT AAAGGAGAAG TTCAAAAACC AAATCAAGCA AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT
CTCAAACTAA ATCAAATGTT CCACCCACTC AAGATGCAGA CACTAAAAGT CCTACTGCAC AACCTGAACA AGCTGAAAAT
TCTGCTCCAA CAGCCGAACA AACTGAATCC CCCGAATTAC AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA
TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAACT
GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGT
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Date: 09 NOV 2017 **Signature:**

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