Certificate of Analysis for MRA-157

Plasmodium falciparum, Strain W2

Catalog No. MRA-157

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Product Description:

Plasmodium falciparum (P. falciparum), strain W2 was cloned from the Indochina III/CDC isolate originally derived from a Laotian patient who failed chloroquine therapy. *P. falciparum*, strain W2 is reported to be resistant to chloroquine and susceptible to mefloquine.

Lot: 70023838[1] **Manufacturing Date: 29MAR2019**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[4]		
Chloroquine	Report results	79.5 ± 1.8 nM
Artemisinin	Report results	2.0 ± 0.1 nM
Quinine	Report results	62.5 ± 2.9 nM
Cycloguanil	Report results	1173 ± 54.0 nM
Pyrimethamine	Report results	17870 ± 1648.2 nM
Sulfadoxine	Report results	295900 ± 27292 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (785 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	4.93%
Total parasitemia	≥ 2%	6.46%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	0.56%
Total parasitemia	≥ 1%	2.53%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[10]	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[2]		
DNA Detection by PCR	None detected	None detected

[1]MRA-157 was produced by cultivation of BEI Resources MR-MRA-157 lot 59210231 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 10 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]Testing completed on vialed post-freeze material

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[3]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.
[4]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].
[5]Primer sequences and conditions for PCR are available upon request.
[6]Testing completed on bulk material prior to vialing and freezing
[7]Parasitemia was determined after 10 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.
[10]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-157 MSP2 Sequence

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TATTAAAAAT GAAAGTAAAT ATAGCAACAC ATTCATAAAC AATGCTTATA ATATGAGTAT AAGGAGAAGT ATGGCAAATG
AAGGTTCTAA TACTACTAGT GTAGGTGCAA ATGCTCCAAA TGCTGATACT ATTGCTAGTG GAAGTCAAAG TAGTACAAAT
AGTGCAAGTA CTAGTACTAC TAATAATGGA GAATCACAAA CTACTACTCC TACCGCTGCT GATACTATTG CTAGTGGAAG
TCAAAGGAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT CACAAACTAC TACTCCTACC GCTGCTGATA
CTATTGCTAG TGGAAGTCAA AGGAGTACAA ATAGTGCAAG TACTAGTACT ACTAATAATG GAGAATCACA AACTACTACT
CCTACCGCTG CTGATACCCC TACTGCTACA GAAAGTAATT CACCTTCACC ACCCATCACT ACTACAGAAA GTTCAAGTTC
TGGCAATGCA CCAAATAAAA CAGACGGTAA AGGAGAAGAG AGTGAAAAAC AAAATGAATT AAATGAATCA ACTGAAGAAG
GACCCAAAGC TCCACAAGAA CCTCAAACGG CAGAAAATGA AAATCCTGCT GCACCAGAGA ATAAAGGTAC AGGACAACAT
GGACATATGC ATGGTTCTAG AAATAATCAT CCACAAAATA CTTCTGATAG TCAAAAAGAA TGTACCGATG GTAACAAAGA
AAACTGTGGA GCAGCAACAT CCCTCTTAAA TAACTCTAGT AATATTGCTT CAATAAATAA ATTTG
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