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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-615

Plasmodium falciparum, Strain W2mef

Catalog No. MRA-615

Product Description:

Plasmodium falciparum (P. falciparum), strain W2mef is a mefloquine-resistant clone derived from clone W2 after *in vitro* passage in mefloquine. Strain W2 was cloned from the Indochina III/CDC isolate originally derived from a Laotian patient who failed chloroquine therapy. MRA-615 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms 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 18 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: 70043270 **Manufacturing Date: 20APR2021**

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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	37.4 ± 0.9 nM
Artemisinin	Report results	5.7 ± 0.3 nM
Quinine	Report results	114.4 ± 5.3 nM
Cycloguanil	Report results	1451 ± 337.1 nM
Pyrimethamine	Report results	26260 ± 1815 nM
Sulfadoxine	Report results	317000 ± 29238 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (18 days post-infection)[3]		
Ring-stage parasitemia	Report results	3.16%
Total parasitemia	≥ 2%	5.38%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.55%
Total parasitemia	≥ 1%	3.03%
Viability (post-freeze; 1 day 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

BEI Resources
www.beiresources.org

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

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TEST	SPECIFICATIONS	RESULTS
Mycoplasma Contamination[1] DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material
[2]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (greater than 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-615 MSP2 Sequence

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ATATTAAAAA TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AATATGAGTA TAAGGAGAAG TATGGCAAAT
GAAGGTTCTA ATACTACTAG TGTAGGTGCA AATGCTCCAA ATGCTGATAC TATTGCTAGT GGAAGTCAAA GTAGTACAAA
TAGTGCAAGT ACTAGTACTA CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC TGATACTATT GCTAGTGGAA
GTCAAAGGAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA CTACTCCTAC CGCTGCTGAT
ACTATTGCTA GTGGAAGTCA AAGGAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACTACTAC
TCCTACCGCT GCTGATACCC CTACTGCTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTTCAAGTT
CTGGCAATGC ACCAAATAAA ACAGACGGTA AAGGAGAAGA GAGTGAAAAA CAAAATGAAT TAAATGAATC AACTGAAGAA
GGACCCAAAG CTCCACAAGA ACCTCAAACG GCAGAAAATG AAAATCCTGC TGCACCAGAG AATAAAGGTA CAGGACAACA
TGGACATATG CATGGTTCTA GAAATAATCA TCCACAAAAT ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG
AAAACTGTGG AGCAGCAACA TCCCTCTTAA ATAACTCTAG TAATATTGCT TCAATAAATA AATTTGTTG
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/Heather Couch/
Heather Couch 05 JUN 2021
Program Manager or designee, ATCC Federal Solutions

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