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SUPPORTING INFECTIOUS DISEASE RESEARCH

Plasmodium falciparum, Strain W2

Catalog No. MRA-157

This reagent is the tangible property of the U.S. Government.

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. MRA-157 was produced by cultivation of BEI Resources seed lot 59210231 in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 μ g/mL hypoxanthine and 5 μ g/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 16 days. Every 1 to 4 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70051093

Manufacturing Date: 25MAR2022

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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 (IC50) by		
SYBR Green I [®] drug sensitivity assay ²		
Chloroquine	Report results	67.9 ± 6.3 nM
Artemisinin	Report results	10.9 ± 0.3 nM
Quinine	Report results	83.0 ± 3.8 nM
Cycloguanil	Report results	1363 ± 94.2 nM
Pyrimethamine	Report results	36170 ± 3336 nM
Sulfadoxine	Report results	367800 ± 33924 nM
Genotypic Analysis ¹		
Sequencing of Merozoite Surface Protein 2 (MSP2)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i>
gene (~ 790 base pairs)		(Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (10 days post-infection) ³		
Ring-stage parasitemia	Report results	3.46%
Total parasitemia	≥ 2%	5.97%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	3.76%
Total parasitemia	≥ 1%	10.50%
Viability (2 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

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¹Testing completed on vialed, post-freeze material

²A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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 <u>Methods in Malaria Research Sixth Edition</u>. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. <u>Methods in Malaria Research Sixth Edition</u> is available on the <u>BEI Resources website</u>.]

³Testing completed on bulk material prior to vialing and freezing

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-157 MSP2 Sequence

TGTCTATTAT AAATTTCTTT ATTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAAACAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAAATGAAG GTTCTAATAC TACTAGTGTA GGTGCAAATG CTCCAAATGC TGATACTATT GCTAGTGGAA GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA CTACTCCTAC CGCTGCTGAT ACTATTGCTA GTGGAAGTCA AAGGAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACTACTAC TCCTACCGCT GCTGATACTA TTGCTAGTGG AAGTCAAAGG AGTACAAATA GTGCAAGTAC TACTAATAAT GGAGAATCAC AAACTACTAC TCCTACCGCT GCTGATACTA ATACCCCTAC TGCTACAAAGA AGTAATTCAC CTTCACCACC CATCACTACT ACAGAAAGTT CAAGTTCTGG CAATGCACCA AATAAAACAG ACGGTAAAGG AGAAGAGAGT GAAAAACAAA ATGAATTAAA TGAATCAACT GAAGAAGGAC CCAAAGCTC ACAAGAACCT CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA AAGGTACAGG ACAACATGGA CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAGAATGT ACCGATGGTA ACAAGAAAA CTGTGGAGCA GCAACATCCC TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTGTTTTA ATTTCAGCAA CACTTGTTTT ATCTTTTG

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