Certificate of Analysis for MRA-1238

Plasmodium falciparum, Strain IPC 4884

Catalog No. MRA-1238

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain IPC 4884 was isolated in 2011 from the blood of a human patient with malaria in Pursat province, western Cambodia. *P. falciparum*, strain IPC 4884 has shown resistance to artemisinin. MRA-1238 was produced by cultivation of the BEI Resources seed lot 62401488 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 every 1 to 3 days for 16 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: 70035105 **Manufacturing Date: 04JUN2020**

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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	27.9 ± 0.6 nM
Artemisinin	Report results	11.2 nM ± 0.5 nM
Quinine	Report results	111.5 nM ± 5.1 nM
Cycloguanil	Report results	534.8 nM ± 24.6 nM
Pyrimethamine	Report results	19100 nM ± 1320.4 nM
Sulfadoxine	Report results	291400 nM ± 26877 nM
Ring-stage Survival Assay (RSA_{0-3h})[3]		
Dihydroartemisinin (DHA)	Report results	6.28%
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 690 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (16 days post-infection)[4]		
Ring-stage parasitemia	Report results	2.96%
Total parasitemia	≥ 2%	5.11%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	1.76%
Total parasitemia	≥ 1%	3.74%
Viability (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[5]	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

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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 (> 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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]
[3]A detailed $RSA_{0\text{-}3h}$ protocol is available on the Worldwide Antimalarial Resistance Network's website.
[4]Testing completed on bulk material prior to vialing and freezing
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1238 MSP2 Sequence

ATGAAAGTAAATATAGCAACACATTCATAAACAATGCTTATAATAAGAGWATAAGGAGAAGTATGGCAAATGAAGGTTCTAATACTACTAGTGTAGG
TGCAAATGCTCCAAATGCTGATACTATTGCTAGTGGAAGTCAAAGKAGTACAAATAGTGCAAGTACTAGTACTACTAATAATGGAGAATCACAAACT
ACTACTCCTACCGCTGCTGATACCCCTACTGCTACAAAAAGTAATTCACCTTCACCACCCATCACTACTACAGAAAGTAATTCACCTTCACCACCCA
TCACTACTACAGAAAGTAATTCACCTTCACCACCCATCACTACTACAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAACAGACGGTAAAGGAGA
AGAGAGTAAAAAAAAAAAATGAATTAAATGAATCAACTGAAGAAGGACCCAAAGCTCCACAAGAACCTCAAACGGCAGAAAATGAAAATCCTGCTGCA
CCAGAGAATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATG
GTAACAAAGAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTCAGCAACACT
TGTTTTATCTTTTGCCATATTCATATAA

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