Product Information Sheet for MRA-1237

Plasmodium falciparum, Strain IPC 3663

Catalog No. MRA-1237

Product Description:

Plasmodium falciparum (*P. falciparum*), strain IPC 3663 was isolated in 2010 from the blood of a human patient with malaria in Pailin province, western Cambodia. MRA-1237 was produced by cultivation of BEI Resources seed lot 62401490 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 µg/mL hypoxanthine and 5 µg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia for 19 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: 70053227 **Manufacturing Date: 21JUN2022**

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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	14.5 ± 0.3 nM
Artemisinin	Report results	19.3 ± 0.9 nM
Quinine	Report results	59.1 ± 2.7 nM
Cycloguanil	Report results	1392 ± 64.1 nM
Pyrimethamine	Report results	16160 ± 745 nM
Sulfadoxine	Report results	498000 ± 34428 nM
Ring-stage Survival Assay (RSA_{0-3h})[3]		
Dihydroartemisinin (DHA)	Report results	1.2%
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 700 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Sequencing of Kelch 13 (K13) gene (~ 1990 base pairs)	Report results	Consistent with *P. falciparum*, strain 3D7. No mutations present (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (19 days post-infection)[4]		
Ring-stage parasitemia	Report results	2.17%
Total parasitemia	≥ 2%	2.98%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	2.85%
Total parasitemia	≥ 1%	3.28%
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[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

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TEST	SPECIFICATIONS	RESULTS
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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-1237 MSP2 Sequence

TTATATGAATAYGGCAAAAGATAAAACAAGTGTTGCTGAAATTTAAAACAACAAATTTATTTATTGAAGCAATATTACTAGAGTTATTTAAGAGGGA
TGTTGCTGCTCCACAGTTTTCTTTGTTACCATCGGTACATTCTTTTTGACTATCAGAAGTATTTTGTGGATGATTATTTCTAGAACCATGCATATGT
CCATGTTGTCCTGTACCTTTATTCTCTGGTGCAGCAGGATTTTCATTTTCTGCCGTTTGAGGTTCTTGTGGAGCTTTGGGTCCTTCTTCAGTTGATT
CATTTAATTCATTTTGTTTTTCACTCTCTTCTCCTTTACCGTCTGTTTTATTTGGTGCATTGCCAGAACTTGAACTTTCTGTAGTAGTGATGGGTGG
TGAAGGTGAATTACTTTCTGTAGTAGTGATGGGTGGTGAAGGTGAATTACTTTCTGTAGTAGTGATGGGTGGTGAAGGTGAATTACTTTTTGTAGCA
GTAGGGGTATCAGCAGCGGTAGGAGTAGTAGTTTGTGATTCTCCATTATTAGTAGTACTAGTACTTGCACTATTTGTACTACTTTGACTTCCACTAG
CAATAGTATCAGCTTTTGGAGCATTTGCACCTACACTAGTAGTATTAGAACCTTCATTTGCCATACTTCTCCTTATACTCTCCATTTAAACATTGTT
TATGAATGTGTTGCTA

Figure 2: MRA-1237 K13 Sequence

TGAGAAAACGGAAAATAATAGTTTCCTTTTAAATAATAGTAGTTATGGAAATGTTAAAGATAGCCTATTAGAATCCATTGATATGAGTGTATTAGAT
TCGAACTTTGATAGTAAAAAAGATTTTTTACCAAGTAATTTATCAAGAACATTTAATAATATGTCTAAAGATAATATAGGAAATAAATATTTAAATA
AATTGTTAAATAAAAAAAAAGATACTATTACAAATGAAAATAATAATATTAATCATAATAATAATAATAATAATCTGACAGCAAATAATATAACTAA
TAATCTTATTAATAATAATATGAATTCTCCATCAATTATGAATACCAACAAAAAAGAGAATTTTTTAGATGCAGCAAATCTTATAAATGATGATTCT
GGATTAAACAATTTAAAAAAATTTTCAACTGTAAATAATGTAAATGATACTTATGAAAAGAAAATTATTGAAACGGAATTAAGTGATGCTAGTGATT
TTGAAAATATGGTAGGTGATTTAAGAATTACATTTATTAATTGGTTAAAAAAGACACAAATGAATTTTATTCGAGAAAAAGATAAATTATTTAAAGA
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AAATCGATAAAATTAAATTAGTATTATATTTAGAAAAAGAAAAATATTATCAAGAATATAAAAATTTTGAGAATGATAAAAAAAAAATTGTTGATGC
AAATATTGCTACTGAAACTATGATTGATATTAATGTTGGTGGAGCTATTTTTGAAACATCTAGACATACCTTAACACAACAAAAAGATTCATTTATA
GAGAAATTATTAAGTGGAAGACATCATGTAACCAGAGATAAACAAGGAAGAATATTCTTAGATAGGGATAGTGAGTTATTTAGAATTATACTTAACT
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ATTCCCATTAGTATTTTGTATAGGTGGATTTGATGGTGTAGAATATTTAAATTCGATGGAATTATTAGATATTAGTCAACAATGCTGGCGTATGTGT
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TTGAAACTGAGGTGTATGATCGTTTAAGAGATGTATGGTATGTTTCAAGTAATTTAAATATACCTAGAAGAAATAATTGTGGTGTTACGTCAAATGG
TAGAATTTATTGTATTGGGGGATATGATGGCTCTTCTATTATACCGAATGTAGAAGCATATGATCATCGTATGAAAGCATGGGTAGAGGTGGCACCT
TTGAATACCCCTAGATCATCAGCTATGTGTGTTGCTTTTGATAATAAAATTTATGTCATTGGTGGAACTAATGGTGAGAGATTAAATTCTATTGAAG
TATATGAAGAAAAAATGAATAAATGGGAACAATTTCCATATGCCTTATTAGAAGCTAGAAGTTCAGGAGCAGCTTTTAATTACCTTAATCAAATATA
TGTTGTTGGAGGTATTGATAATGAACATAACATATTAGATTCCGTTGAACAATATCAACCATTTAATAAAAGATGGCAATTTCTAAATGGTGTACCA
GAGAAAAAAAATGAATTTTGGAGCTGCCACATTGTCAGATTCTTATATAATTACAGGAGGAGAAAATGGCGAAGTTCTAAATTCATGTCATTTCTTTT
CACCAGATACAAATGAATGGCAGCTTGGCCCATCTTTATTAGTTCCCAGATTT

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