

***Plasmodium falciparum*, Strain K1**

Catalog No. MRA-159

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain K1 was isolated in Thailand and is reported to be a multidrug-resistant strain. MRA-159 was produced by cultivation of BEI Resources seed material 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 14 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: 70043125

Manufacturing Date: 11JUN2021

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I® drug sensitivity assay ²		
Chloroquine	Report results	78.8 ± 7.3 nM
Artemisinin	Report results	5.3 ± 0.2 nM
Quinine	Report results	108.7 ± 7.5 nM
Cycloguanil	Report results	1143 ± 158.4 nM
Pyrimethamine	Report results	28980 ± 1335 nM
Sulfadoxine	Report results	384300 ± 44342 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	≥ 95% sequence identity to <i>P. falciparum</i> , strain K1 (GenBank: ABGV01000272)	98.9% sequence identity to <i>P. falciparum</i> , strain K1 (GenBank: ABGV01000272) (Figure 1)
Functional Activity by PCR Amplification¹ MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (14 days post-infection) ³		
Ring-stage parasitemia	Report results	3.41%
Total parasitemia	≥ 2%	4.88%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	1.18%
Total parasitemia	≥ 1%	2.84%
Viability (post-freeze; 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 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 Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vial, 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 *Methods in Malaria Research Sixth Edition*. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. Available at: to <https://www.beiresources.org/Publications/MethodsInMalariaResearch.aspx>.]

³Testing completed on bulk material prior to vialing and freezing

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-159 MSP2 Sequence

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TTTATTGAAG CAAATATTAC TAGAGTTATT TAAGAGGGAT GTTGCTSCYC CWCRCGKTTTC TTTGTTACCA TCGGTACATT
CTTTTTGACT ATCAGAAGTA TTTTGTGGAT GATTATTTCT AGAACCATGC ATATGTCCAT GTTGTCCTGT ACCTTTATTC
TCTGGTGCAG CAGGATTTTC ATTTTCTGCC GTTTGAGGTT CTTGTGGAGC TTTGGGTCCCT TCTTCAGTTG ATTCATTTAA
TTCATTTTGT TTTTCACTCT CTTCTCCTTT ACCGTCGTGT TTATTTGGTG CATTGCCAGA ACTTGAACCTT TCTGTAGTAG
TGATGGGTGG TGAACGTGAA TTTACTTTCTG TAGTAGTGAT GGGTGGTGAA CGTGAATTAC TTTCTGTAGT AGTGATGGGT
GGTGAACGTG AATTACTTTC TGTAGTAGTG ATGGGTGGTG AACGTGAATT ACTTTCTGTA GTAGTGATGG GTGGTGAACG
TGAATTACTT TCTGTAGCAG TAGGGGTATC AGCAGCGGTA GGAGTAGTAG TTTGTGATTC TCCATTATTA GTAGTACTAG
TACTTGCCTT ATTTGTACTA CTTTACTTTC CACTAGCAAT AGTATCAGCT TTTGGAGCAT TTGCACCTAC ACTCTTAGTA
TTAGAACCTT CATTTGCCAT ACTTCTCCTT ATACTCATAT TATAAGCATT GTTTATGAAT GTGTTGCTAT ATTTACTTTC
ATTTTTAATA TTAAAGGTAA CAAAAATAAA GAAAATTTAT AATAGACAAT GTTTTA
    
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Program Manager or designee, ATCC Federal Solutions

14 JAN 2022

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