

**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.

**Lot<sup>1</sup>: 70022010**

**Manufacturing Date: 25JAN2019**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>2,3</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)<sup>2</sup></b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR green I <sup>®</sup> drug sensitivity assay <sup>4</sup> Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	139.5 ± 12.9 nM 8.0 ± 0.2 nM 193.9 ± 4.5 nM 774.3 ± 35.7 nM 30810 ± 709.5 nM 580000 ± 26720 nM
<b>Genotypic Analysis<sup>2</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 420 base pairs)	≥ 99% sequence identity to <i>P. falciparum</i> , strain K1 (GenBank: ABGV01000272.1)	100% sequence identity to <i>P. falciparum</i> , strain K1 (GenBank: ABGV01000272.1) (Figure 1)
<b>Functional Activity by PCR Amplification<sup>2</sup></b> MSP2 PCR amplicon analysis <sup>5</sup>	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
<b>Level of Parasitemia</b> Pre-freeze <sup>6,7</sup> Ring-stage parasitemia Total parasitemia Post-freeze <sup>2,8</sup> Ring-stage parasitemia Total parasitemia	Report results ≥ 2%  Report results ≥ 1%	3.27% 4.63%  4.13% 5.51%
<b>Viability<sup>2,9</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells (Figure 2)
<b>Sterility (22-day incubation)<sup>2</sup></b> Harpo's HTYE broth <sup>10</sup> , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination<sup>2</sup></b> DNA Detection by PCR	None detected	None detected

<sup>1</sup>MRA-159 was produced by cultivation of BEI Resources MR-MRA-159 lot 63527536 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<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia daily for 15 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.

<sup>2</sup>Testing completed on vial post-freeze material

<sup>3</sup>Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.

<sup>4</sup>A SYBR Green I<sup>®</sup> anti-malarial drug sensitivity assay in 96-well plates was used to determine IC<sub>50</sub> 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<sup>®</sup>-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>].

<sup>5</sup>Primer sequences and conditions for PCR are available upon request.

<sup>6</sup>Testing completed on bulk material prior to vialing and freezing

<sup>7</sup>Parasitemia was determined after 15 days post infection by microscopic counts of Giemsa-stained blood smears.

<sup>8</sup>Parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

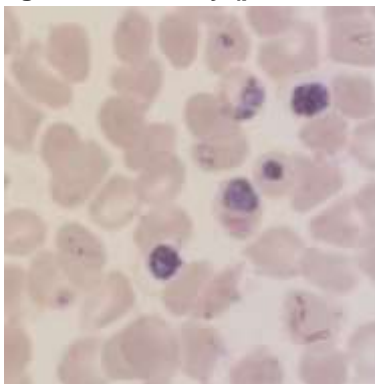
<sup>9</sup>Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.

<sup>10</sup>Atlas, 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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AACTACTACT CCTACCGCTG CTGATACCCC TACTGCTACA GAAAGTAATT CACGTTTACC ACCCATCACT ACTACAGAAA
GTAATTCACG TTCACCACCC ATCACTACTA CAGAAAGTAA TTCACGTTCA CCACCCATCA CTACTIONACA AAGTAATTCA
CGTTCACCAC CCATCACTAC TACAGAAAGT AATTCACGTT CACCACCCAT CACTACTACA GAAAGTTCAA GTTCTGGCAA
TGCACCAAAT AAAACAGACG GTAAAGGAGA AGAGAGTGAA AAACAAAATG AATTAAATGA ATCAACTGAA GAAGGACCCA
AAGCTCCACA AGAACCTCAA ACGGCAGAAA ATGAAAATCC TGCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT
ATGCATGGTT CTAGA
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**Figure 2: Viability (post-freeze)**



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Heather Couch

31 MAY 2019

Program Manager or designee, ATCC Federal Solutions

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