b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-159

Plasmodium falciparum, Strain K1

Catalog No. MRA-159

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

Product Description: *Plasmodium falciparum (P. falciparum)*, strain K1 was isolated in Thailand and is reported to be a multidrug-resistant strain.

Lot[1]: 63433330 **Manufacturing Date: 21MAY2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2]		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[4]		
Chloroquine	Report results	181.5 ± 8.4 nM
Artemisinin	Report results	3.4 ± 0.1 nM
Quinine	Report results	130.6 ± 3.0 nM
Cycloguanil	Report results	962.1 ± 22.2 nM
Pyrimethamine	Report results	25640 ± 1181.2 nM
Sulfadoxine	Report results	508000 ± 11698.2 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 540 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain K1 (GenBank: ABGV01000272)	100% sequence identity to *P. falciparum*, strain K1 (GenBank: ABGV01000272) (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	3.84%
Total parasitemia	≥ 2%	5.76%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	0.33%
Total parasitemia	≥ 1%	3.61%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[10], 37°C and 26°C, aerobic	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose 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[2]		
DNA Detection by PCR	None detected	None detected

[1]MRA-159 was produced by cultivation of BEI Resources MR-MRA-159 lot 58278723 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 daily for 11 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.

[2]Testing completed on vialed post-freeze material

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[3]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 4 days.
[4]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. Available at: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx].
[5]Primer sequences and conditions for PCR are available upon request.
[6]Testing completed on bulk material prior to vialing and freezing
[7]Parasitemia was determined after 11 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[10]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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AATTTATTTA TTGAAGCAAT ATTACTAGAG TTATTTAAGA GGGATGTTGC TGCTCCACAG TTTTCTTTGT TACCATCGGT
ACATTCTTTT TGACTATCAG AAGTATTTTG TGGATGATTA TTTCTAGAAC CATGCATATG TCCATGTTGT CCTGTACCTT
TATTCTCTGG TGCAGCAGGA TTTTCATTTT CTGCCGTTTG AGGTTCTTGT GGAGCTTTGG GTCCTTCTTC AGTTGATTCA
TTTAATTCAT TTTGTTTTTC ACTCTCTTCT CCTTTACCGT CTGTTTTATT TGGTGCATTG CCAGAACTTG AACTTTCTGT
AGTAGTGATG GGTGGTGAAC GTGAATTACT TTCTGTAGTA GTGATGGGTG GTGAACGTGA ATTACTTTCT GTAGTAGTGA
TGGGTGGTGA ACGTGAATTA CTTTCTGTAG TAGTGATGGG TGGTGAACGT GAATTACTTT CTGTAGTAGT GATGGGTGGT
GAACGTGAAT TACTTTCTGT AGCAGTAGGG GTATCAGCAG CGGTAGGAGT AGTAGTT
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/Heather Couch/
Heather Couch 31 MAY 2019
Program Manager or designee, ATCC Federal Solutions

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