b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-925

Plasmodium falciparum, Strain GB4

Catalog No. MRA-925

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

Product Description: *Plasmodium falciparum (P. falciparum)*, strain GB4 was cloned in 2000 from the Ghana III/CDC strain, originally isolated by the Centers for Disease Control and Prevention from a patient (hospitalized in Georgia, USA) who had acquired the infection in Ghana.

Lot[1]: 70006878 **Manufacturing Date: 22SEP2017**

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	24.9 ± 1.1 nM
Artemisinin	Report results	10.9 ± 0.3 nM
Quinine	Report results	72.7 ± 3.3 nM
Cycloguanil	Report results	9.9 ± 0.7 nM
Pyrimethamine	Report results	42.1 ± 3.9 nM
Sulfadoxine	Report results	553400 ± 38258 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 590 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	2.46%
Total parasitemia	≥ 2%	3.38%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	1.66%
Total parasitemia	≥ 1%	3.56%
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-925 was produced by cultivation of BEI Resources MR-MRA-925 lot 58422569 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 9 days. Every 1 to 2 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 9 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze 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-925 MSP2 Sequence

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TTAAAAATGA AAGTAAATAT AGCAACACAT TCATAAACAA TGCTTATMAT ATGAGTATAA GGAGAAGTAT GGCAGAAAGT
AAGACTCCTA CTGGTACTGG TGCTAGTGGT AATCCTCCTG CTGGTGCTGG TGCTAGTGGT AATCCTCCTG CTGGTGCTGG
TGCTAGTGGT AATCCTCCTG CTGGTGCTGG TGCTAGTGGT AATCCTCCTG CTGGTGCTGG TGCTAGTGGT AATCCTCCTG
CTGGTGCTGG TGCTAGTGGT AATCCTCCTG CTGGTGCTGA GAGAAGTCCA AGTACTACCA CAACTACCAC AACTACCACA
ACTACTAATG ATGCAGAAGC ATCTACCAGT ACCTCTTCAG AAAATCCAAA TCATAATAAT GCCAAAACAA ATCCAAAAGG
TAATGGAGGA GTTCAAGAAC CAAATAAAGC AAATACAGAA ACTCAAAATA ACTCAAATGT TCAACAAGAC TCTCAAACTA
AATCAAATGT TCCACCCACT CAAGATGCAG ACACTAAAAG TCCTACTGCA CAACCTGAAC AAGCTGAAAA TTCTGCTCCA
ACAGCCGAAC AAACTGAATC CCCCGAATTA CAATCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG
TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA CAAAGAAAAC TGTGGAGCAG
CAACATCCCT CTTAAATAAC TCTAGTAATA TTGCTTCAAT AAATAAAT
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/Heather Couch/
Heather Couch 29 AUG 2018
Program Manager or designee, ATCC Federal Solutions

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