Certificate of Analysis for MRA-733

Plasmodium falciparum, Strain FCC-2/Hainan

Catalog No. MRA-733

Product Description: *Plasmodium falciparum (P. falciparum)*, strain FCC-2/Hainan was isolated in 1979 from the blood of a human patient in Hainan Island, China. MRA-733 was derived from ATCC® 30993™, which was deposited at ATCC® by W. Trager. *P. falciparum*, strain FCC-2/Hainan was identified as sensitive to chloroquine.

Lot[1]: 70005221 **Manufacturing Date: 16JUN2017**

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	7.2 ± 0.2 nM
Artemisinin	Report results	15.6 ± 0.4 nM
Quinine	Report results	31.7 ± 1.5 nM
Cycloguanil	Report results	138.0 ± 6.4 nM
Pyrimethamine	Report results	7644 ± 352.1 nM
Sulfadoxine	Report results	449700 ± 20716.8 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 700 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain FCC-2/Hainan (GenBank: ABGW01004745.1)	99.9% sequence identity to *P. falciparum*, strain FCC-2/Hainan (GenBank: ABGW01004745.1)
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	2.68%
Total parasitemia	≥ 2%	4.38%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	0.84%
Total parasitemia	≥ 1%	5.07%
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-733 was produced by cultivation of BEI Resources MRA-733 lot 3872735 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%

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CO_2, 5% O_2) and monitored for parasitemia daily for 8 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.

[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 1 day.

[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 8 days post infection by microscopic counts of Giemsa-stained blood smears.

[8]Parasitemia was determined after 1 day post infection by microscopic counts of Giemsa-stained blood smears.

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 1 day 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-733 MSP2 Sequence

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TTTGTTACCT TTAATATTAA AAATGAAAGT AAATATAGCA ACACATTCAT AAACAATGCT TATAATATGA GTATAAGGAG
AAGTATGGCA AATGAAGGTT CTAATACTAA TAGTGTAGAT GCAAAAGCTC CAAAAGCTGA TACTATTGCT AGTGGAAGTC
AAAGTAGTAC AAATAGTGCA AGTACTAGTA CTACTAATAA TGGAGAATCA CAAACTACTA CTCCTACCGC TGCTGATACT
ATTGCTAGTG GAAGTCAAAG GAGTACAAAT AGTGCAAGTA CTAGTACTAC TAATAATGGA GAATCACAAA CTACTACTCC
TACCGCTGCT GATACCCCTA CTGCTACAGA AAGTAATTCA CCTTCACCAC CCATCACTAC TACAGAAAGT TCAAGTTCTG
GCAATGCACC AAATAAAACA GACGGTAAAG GAGAAGAGAG TGAAAAACAA AATGAATTAA ATGAATCAAC TGAAGAAGGA
CCCAAAGCTC CACAAGAACC TCAAACGGCA GAAAATGAAA ATCCTGCTGC ACCAGAGAAT AAAGGTACAG GACAACATGG
ACATATGCAT GGTTCTAGAA ATAATCATCC ACAAAATACT TCTGATAGTC AAAAAGAATG TACCGATGGT AACAAAGAAA
ACTGTGGAGC AGCAACATCC CTCTTAAATA ACTCTAGTAA TATTGCTTCA ATAAATAAAT T
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/Heather Couch/
Heather Couch 03 OCT 2018
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