b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-309

Plasmodium falciparum, Strain FCB

Catalog No. MRA-309

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain FCB originated in Southeast Asia and has shown resistance to chloroquine.

Lot[1]: 63901356 **Manufacturing Date: 30NOV2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	28.3 ± 1.3 nM
Artemisinin	Report results	10.2 ± 0.9 nM
Quinine	Report results	166.7 ± 23.1 nM
Cycloguanil	Report results	782.5 ± 126.7 nM
Pyrimethamine	Report results	32.8 ± 1.5 nM
Sulfadoxine	Report results	553200 ± 128508 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 800 base pair amplicon (Figure 2)
Level of Parasitemia		
Pre-freeze[5]	Report results	4.8%
Post-freeze[6]	> 1%	11.39%
Viability (post-freeze)[7]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)		
Harpo's HTYE broth[8], 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		
DNA Detection by PCR	None detected	None detected

[1]MRA-309 was produced by cultivation of the deposited 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 12 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% hematocrit.

[2]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.

[3]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.mr4.org/Publications/MethodsinMalariaResearch.aspx].

[4]Primer sequences and conditions for PCR are available upon request.

[5]Pre-freeze parasitemia was determined after 12 days post infection by microscopic counts of Giemsa-stained blood smears.

[6]Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

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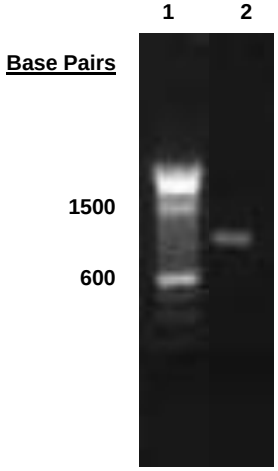
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-309 MSP2 Sequence

ATAAATTTCT TTATTTTTGT TACCTTTAAT ATTAAAAATG AAAGTAAATA TAGCAACACA TTCATAAACA ATGCTTATAA
TATGAGTATA AGGAGAAGTA TGACAGAAAG TAATCCTCCT ACTGGTGCTA GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA
GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT
GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTTC TGGTGATGGT AATGGTGCTA ATCCTGGTGC
AGATGCTGAG AGAAGTCCAA GTACTCCCGC TACTACCACA ACTACCACAA CTACTAATGA TGCAGAAGCA TCTACCAGTA
CCTCTTCAGA AAATCCAAAT CATAATAATG CCGAAACAAA TCAAGCAAAT AAAGAAACTC AAAATAACTC AAATGTTCAA
CAAGACTCTC AAACTAAATC AAATGTTCCA CCCACTCAAG ATGCAGACAC TAAAAGTCCT ACTGCACAAC CTGAACAAGC
TGAAAATTCT GCTCCAACAG CCGAACAAAC TGAATCCCCC GAATTACAAT CTGCACCAGA GAATAAAGGT ACAGGACAAC
ATGGACATAT GCATGGTTCT AGAAATAATC ATCCACAAAA TACTTCTGAT AGTCAAAAAG AATGTACCGA TGGTAACAAA
GAAAACTGTG GAGCAGCAAC ATCCCTCTTA AATAACTCTA GTAATATTGC TTCAAT

Figure 2: PCR Amplification of MSP2



Lane 1: 100 base pair ladder
Lane 2: 100 ng of MRA-309

Date: 17 FEB 2016 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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