Certificate of Analysis for MRA-909

Plasmodium falciparum, Strain FCR-1/FVO

Catalog No. MRA-909

Product Description: *Plasmodium falciparum (P. falciparum)*, strain FCR-1/FVO started at the Rockefeller University in 1976, by continuous culture of the Vietnam-Oak Knoll (FVO) strain. The parent *P. falciparum* FVO strain, which is chloroquine-resistant, was originally isolated from a patient who returned from Vietnam and was hospitalized at the Oak Knoll Naval Hospital, Oakland, California, USA. MRA-909 was derived from ATCC® 30930™, which was deposited at ATCC® by W. Trager. *P. falciparum*, strain FCR-1/FVO was deposited as chloroquine-resistant.

Lot[1]: 70011949 **Manufacturing Date: 06FEB2018**

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	55.7 ± 2.6 nM
Artemisinin	Report results	11.6 ± 0.5 nM
Quinine	Report results	289.5 ± 26.7 nM
Cycloguanil	Report results	1013.0 ± 93.4 nM
Pyrimethamine	Report results	30.6 ± 3.5 nM
Sulfadoxine	Report results	23750 ± 5517 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 680 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	2.15%
Total parasitemia	≥ 2%	4.65%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	2.06%
Total parasitemia	≥ 1%	2.94%
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-909 was produced by cultivation of BEI Resources MR-MRA-909 lot 58218068 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,

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5% CO_2, 5% O_2) and monitored for parasitemia daily for 8 days. Every 2 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 3 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 8 days post infection by microscopic counts of Giemsa-stained blood smears.

[8]Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 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-909 MSP2 Sequence

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TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AATATGAGTA TAAGGAGAAG TATGACAGAA AGTAATCCTC
CTACTGGTGC TAGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT
GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG
TAGTGCTGGT TCTGGTGATG GTAATGGTGC TAATCCTGGT GCAGATGCTG AGAGAAGTCC AAGTACTCCC GCTACTACCA
CAACTACCAC AACTACTAAT GATGCAGAAG CATCTACCAG TACCTCTTCA GAAAATCCAA ATCATAATAA TGCCGAAACA
AATCAAGCAA ATAAAGAAAC TCAAAATAAC TCAAATGTTC AACAAGACTC TCAAACTAAA TCAAATGTTC CACCCACTCA
AGATGCAGAC ACTAAAAGTC CTACTGCACA ACCTGAACAA GCTGAAAATT CTGCTMCAAC AGCCGAACAA ACTGAATCCC
CCGAATTMCA ATCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT ATGCATGGTT CTAGAAATAA TCATCCACAA
AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGA
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/Heather Couch/
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