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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-732

Plasmodium falciparum, Strain FCR-8/West African

Catalog No. MRA-732

Product Description:

Plasmodium falciparum (P. falciparum), strain FCR-8/West African was originally isolated from the blood of a human patient collected in 1978 in West Africa. MRA-732 was derived from ATCC® 50028™, which was deposited to ATCC® by W. Trager. *P. falciparum*, strain FCR-8/West African was identified as sensitive to chloroquine. MRA-732 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per 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 for 23 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.

Lot: 70037555 **Manufacturing Date: 16AUG2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	12.9 ± 0.3 nM
Artemisinin	Report results	9.7 ± 0.2 nM
Quinine	Report results	51.0 ± 4.7 nM
Cycloguanil	Report results	13.6 ± 2.8 nM
Pyrimethamine	Report results	52.0 ± 10.9 nM
Sulfadoxine	Report results	490300 ± 45222 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (825 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (23 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.79%
Total parasitemia	2% or greater	5.26%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	1.21%
Total parasitemia	1% or greater	1.93%
Viability (post-freeze; 2 days post-infection)[1]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[4]	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud 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[1]		
DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material

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[2]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (greater than 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.]
[3]Testing completed on bulk material prior to vialing and freezing
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-732 MSP2 Sequence

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TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA ATAGTGTAGG TGCAAATGCT
GATACTATTG CTAGTGGAAG TCAAAGGAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT CACAAACTAC
TACTCCTACC GCTGCTGATA CTATTGCTAG TGGAAGTCAA AGGAGTACAA ATAGTGCAAG TACTAGTACT ACTAATAATG
GAGAATCACA AACTACTACT CCTACCGCTG CTGATACTAT TGCTAGTGGA AGTCAAAGGA GTACAAATAG TGCAAGTACT
AGTACTACTA ATAATGGAGA ATCACAAACT ACTACTCCTA CCGCTGCTGA TACCCCTACT GCTACAGAAA GTTCAAGTTC
TGGCAATGCA CCAAATAAAA CAGACGGTAA AGGAGAAGAG AGTGAAAAAC AAAATGAATT AAATGAATCA ACTGAAGAAG
GACCCAAAGC TCCACAAGAA CCTCAAACGG CAGAAAATGA AAATCCTGCT GCACCAGAGA ATAAAGGTAC AGGACAACAT
GGACATATGC ATGGTTCTAG AAATAATCAT CCACAAAATA CTTCTGATAG TCAAAAAGAA TGTACCGATG GTAACAAAGA
AAACTGTGGA GCAGCAACAT CCCTCTTAAA TAACTCTAGT AATATTGCTT CAATAAATAA ATTTGTTGTT TTAATTTCAG
CAACACTTGT TTTATCTTTT GCCAT
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/Heather Couch/
Heather Couch 13 FEB 2021
Program Manager or designee, ATCC Federal Solutions

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