Certificate of Analysis for MRA-736

Plasmodium falciparum, Strain FCR-3/FMG (Gambia)

Catalog No. MRA-736

Product Description: *Plasmodium falciparum (P. falciparum)*, strain FCR-3/FMG (Gambia) was originally isolated in 1976 from the blood of a human patient collected in The Gambia, West Africa. MRA-736 was derived from ATCC® 30932™, which was deposited at ATCC® by W. Trager.

Lot[1]: 70017000 **Manufacturing Date: 20JUL2018**

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	55.3 ± 6.4 nM
Artemisinin	Report results	7.6 ± 0.7 nM
Quinine	Report results	131.9 ± 12.2 nM
Cycloguanil	Report results	588.1 ± 40.7 nM
Pyrimethamine	Report results	141.7 ± 19.6 nM
Sulfadoxine	Report results	276800 ± 38363 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 480 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	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	2.02%
Total parasitemia	≥ 2%	3.56%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	1.88%
Total parasitemia	≥ 1%	3.75%
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-736 was produced by cultivation of BEI Resources MR-MRA-736 lot 58319489 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 14 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 5 days.

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[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 14 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 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-736 MSP2 Sequence

TTTTTGTTAC CTTTAATATT AAAAATGAAA GTAAATATAG CAACACATTC ATAAACAATG CTTATAATAT GAGTATAAGG
AGAAGTATGA CAGAAAGTAA TCCTCCTACT GGTGCTAGTG GTAGTGCTGG TGGTAGTGCT GGTGGTAGTG CTGGTGGTAG
TGCTGGTGGT AGTGCTGGTG GKAGTGCTGG TGGTAGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTGGW AGTGCTGGTG
GKAGTGCTGG KGGTAGTGCT GGTGGTAGTG CTGGTTCTGG TGATGGTAAT GGTGCTAATC CTGGTGCARA TGCTGAGAGA
AGTCCAAGTA CTCCCGCTAC TACCACAACT ACCACAACTA CTAATGATGC WKAAGCATCT ACCARTACCT CTTCAGAAAA
TCCAAATCAT AATAATGCCK AAACAAATCA AGCAAATAAA GAAACTCAAA ATAACTCAAA TGTTCAACAA GACTCTCAAA
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/Heather Couch/
Heather Couch 23 SEP 2018
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