Certificate of Analysis for MRA-1257

Plasmodium falciparum, Strain AM1

Catalog No. MRA-1257

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain AM1 is a derivative of *P. falciparum*, strain 3D7 (available as BEI Resources MRA-102) that was selected for fosmidomycin resistance and cloned by limiting dilution. *P. falciparum*, strain 3D7 was cloned from *P. falciparum*, strain NF54 (available as BEI Resources MRA-1000) by limiting dilution; the original NF54 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands. *P. falciparum*, strain AM1 is resistant to fosmidomycin (FSM^R), reportedly a result of the loss of haloacid dehalogenase (PfHAD1) function.

Lot[1]: 63459854 **Manufacturing Date: 23JUN2015**

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 Artemisinin Fosmidomycin	 Report results Report results Report results	 6.4 ± 0.3 nM 17.1 ± 0.4 nM 5817 ± 402 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 base pairs) MSP2 PCR amplicon analysis[5]	 Consistent with *P. falciparum* ~ 600-900 base pair amplicon	 Consistent with *P. falciparum* (Figure 1)[4] ~ 850 base pair amplicon
Level of Parasitemia Pre-freeze[6] Post-freeze[7]	 Report results > 1%	 2.89% 1.26%
Viability (post-freeze)[8]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation) Harpo's HTYE broth[9], 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	 No growth No growth No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination DNA Detection by PCR	 None detected	 None detected

[1]MRA-1257 was produced by cultivation of the deposited material in fresh human erythrocytes 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 49 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to 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 2 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].

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[4]100% sequence identity to GenBank: AE001362 (*P. falciparum*, strain 3D7)
[5]Primer sequences and conditions for PCR are available upon request.
[6]Pre-freeze parasitemia was determined after 49 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Post-freeze parasitemia was determined after 2 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Viability was confirmed by examination of infected erythrocytes for parasitemia at 2 days post infection.
[9]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1257 MSP2 Sequence

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TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AGAAAGTAAG CCTTCTACTG GTGCTGGTGG TAGTGCTGGT
GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT TCTGGTGATG GTAATGGTGC
AGATGCTGAG GGAAGTTCAA GTACTCCCGC TACTACCACA ACTACCAAAA CTACCACAAC TACCACAACT ACTAATGATG
CAGAAGCATC TACCAGTACC TCTTCAGAAA ATCCAAATCA TAAAAATGCC GAAACAAATC CAAAAGGTAA AGGAGAAGTT
CAAGAACCAA ATCAAGCAAA TAAAGAAACT CAAAATAACT CAAATGTTCA ACAAGACTCT CAAACTAAAT CAAATGTTCC
ACCCACTCAA GATGCAGACA CTAAAAGTCC TACTGCACAA CCTGAACAAG CTGAAAATTC TGCTCCAACA GCCGAACAAA
CTGAATCCCC CGAATTACAA TCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA TGCATGGTTC TAGAAATAAT
CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT GGAGCAGCAA CATCCCTCTT
AAATAACTCT AGTAATATTG CTTCAATAAA TAAATTTGTT GTTTTAATTT CAGCAACACT TGTTTTATCT TTTG
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Date: 08 APR 2016 **Signature:**

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