Certificate of Analysis for MRA-1258

Plasmodium falciparum, Strain AM1_Hsp110:PfHAD1-GFP

Catalog No. MRA-1258

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain AM1_Hsp110:PfHAD1-GFP is a *P. falciparum*, strain AM1 derivative that was created by transfection of the parent strain with a plasmid used to express a haloacid dehalogenase (PfHAD1) in fosmidomycin (FSM) resistant strains, and contains a carboxyl-terminal green fluorescent protein (GFP). *P. falciparum*, strain AM1_Hsp110:PfHAD1-GFP is reported to be more sensitive to FSM than the parent *P. falciparum*, strain AM1 (available as BEI Resources MRA-1257).

Lot[1]: 63597674 **Manufacturing Date: 07JUL2015**

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	 6.2 ± 0.1 nM 16.0 ± 0.4 nM 1066 ± 49.1 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 base pairs) MSP2 PCR amplicon analysis[4]	 Consistent with *P. falciparum* ~ 600-900 base pair amplicon	 Consistent with *P. falciparum* (Figure 1) ~ 900 base pair amplicon
Phenotypic Analysis GFP expression[5]	 Positive	 Positive (Figure 2)
Level of Parasitemia Pre-freeze[6] Post-freeze[7]	 Report results > 1%	 2.85% 2.60%
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-1258 was produced by cultivation of MRA-1258 lot 63459858 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 6 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. MRA-1258 lot 63459858 was treated and cleared of mycoplasma contamination prior to production of this lot.

[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

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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]GFP expression was measured using an Olympus microscope at 100x magnification.

[6]Pre-freeze parasitemia was determined after 6 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-1258 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 TTTGC
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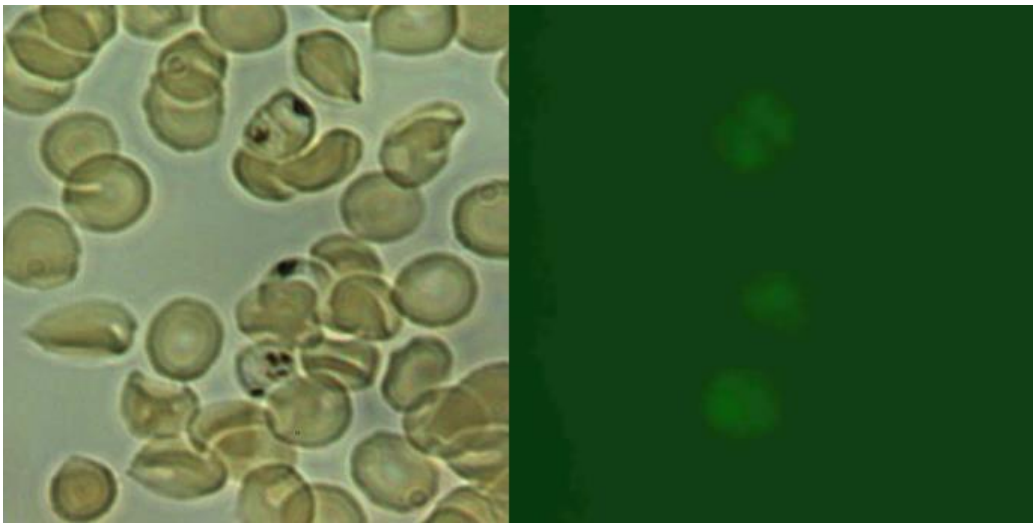
Figure 2: GFP Expression by MRA-1258



Date: 07 APR 2016 **Signature:**

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