Certificate of Analysis for MRA-150

Plasmodium falciparum, Strain Dd2

Catalog No. MRA-150

Product Description: *Plasmodium falciparum (P. falciparum)*, strain Dd2 is a clone derived from W2-MEF, which was selected from W2-MCII after 6 months of continuous cultivation in the presence of mefloquine. W2-MCII was derived from W2'82 after 12 months of continuous cultivation in the presence of mefloquine, which was itself derived from Indochina III/CDC. *P. falciparum*, strain Dd2 was deposited as resistant to chloroquine, pyrimethamine and mefloquine.

Lot[1]: 70008762 **Manufacturing Date: 06SEP2017**

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	Report results	50.0 ± 1.2 nM
Artemisinin	Report results	23.5 ± 0.5 nM
Quinine	Report results	226.7 ± 10.4 nM
Cycloguanil	Report results	1483 ± 171.1 nM
Pyrimethamine	Report results	28300 ± 2610.2 nM
Sulfadoxine	Report results	349000 ± 48370 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain Dd2 (GenBank: AASM01000018.1)	100% sequence identity to *P. falciparum*, strain Dd2 (GenBank: AASM01000018.1) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]		
Ring-stage parasitemia	Report results	5.20%
Total parasitemia	≥ 2%	5.75%
Post-freeze[6]		
Ring-stage parasitemia	Report results	0.40%
Total parasitemia	≥ 1%	2.40%
Viability (post-freeze)[7]	Growth in infected red blood cells	Growth in infected red blood cells (Figure 2)
Sterility (21-day incubation)		
Harpo's HTYE broth[8], 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		
DNA Detection by PCR	None detected	None detected

[1]MRA-150 was produced by cultivation of MR-MRA-150 lot 64043571 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)

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and monitored for parasitemia daily for 8 days. Every 1 to 2 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for 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 3 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.beiresources.org/Publications/MethodsinMalariaResearch.aspx].

[4]Primer sequences and conditions for PCR are available upon request.

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

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

[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 days post infection.

[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-150 MSP2 Sequence

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TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC
AAATGAAGGT TCTAATACTA CTAGTGTAGG TGCAAATGCT CCAAATGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA
CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC ACAAACTACT ACTCCTACCG CTGCTGATAC TATTGCTAGT
GGAAGTCAAA GGAGTACAAA TAGTGCAAGT ACTAGTACTA CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC
TGATACTATT GCTAGTGGAA GTCAAAGGAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA
CTACTCCTAC CGCTGCTGAT ACCCCTACTG CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA
AGTTCTGGCA ATGCACCAAA TAAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA
AGAAGGACCC AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC
AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC
AAAGAAAACT GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTT
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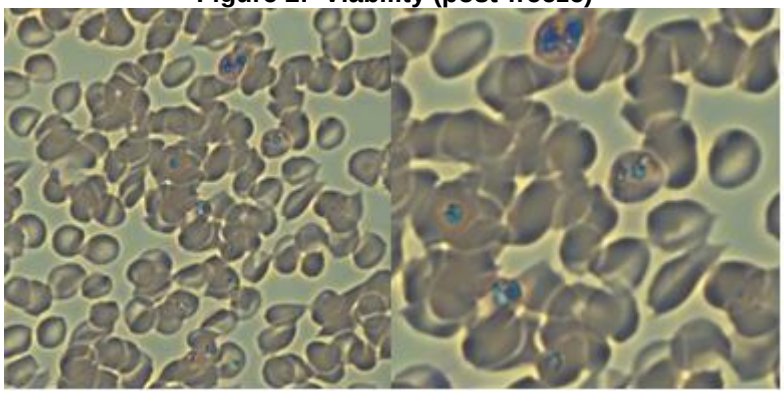
Figure 2: Viability (post-freeze)



Date: 18 DEC 2017 **Signature:**

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