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]: 62781319 **Manufacturing Date: 15JUL2014**

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 Quinine Cycloguanil Pyrimethamine Sulfadoxine	 Report results Report results Report results Report results Report results Report results	 43.6 ± 3.0 nM 6.1 ± 0.4 nM 100.1 ± 13.9 nM 1489 ± 710.2 nM 17960± 2907.4 nM 546600 ± 37787.8 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs) MSP2 PCR amplicon analysis[4]	 ≥ 99% sequence identity to *P. falciparum*, strain Dd2 (GenBank: AASM01000018.1) ~ 600-900 base pair amplicon	 100% sequence identity to *P. falciparum*, strain Dd2 (GenBank: AASM01000018.1) (Figure 1) ~ 800 base pair amplicon
Level of Parasitemia Pre-freeze[5] Post-freeze[6]	 Report results > 1%	 4.24% 8.13%
Viability (post-freeze)[7]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation) Harpo's HTYE broth[8], 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-150 was produced by cultivation of MR-MRA-150 Lot 58410301 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 12 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 1.14% 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 4 days.

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[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: to https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx.
[4]Primer sequences and conditions are available upon request.
[5]Pre-freeze parasitemia was determined after 12 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 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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TATTTATTGA AGCAATATTA CTAGAGTTAT TTAAGAGGGA TGTTGCTGCT CCACAGTTTT CTTTGTTACC ATCGGTACAT
TCTTTTTGAC TATCAGAAGT ATTTTGTGGA TGATTATTTC TAGAACCATG CATATGTCCA TGTTGTCCTG TACCTTTATT
CTCTGGTGCA GCAGGATTTT CATTTTCTGC CGTTTGAGGT TCTTGTGGAG CTTTGGGTCC TTCTTCAGTT GATTCATTTA
ATTCATTTTG TTTTTCACTC TCTTCTCCTT TACCGTCTGT TTTATTTGGT GCATTGCCAG AACTTGAACT TTCTGTAGTA
GTGATGGGTG GTGAAGGTGA ATTACTTTCT GTAGCAGTAG GGGTATCAGC AGCGGTAGGA GTAGTAGTTT GTGATTCTCC
ATTATTAGTA GTACTAGTAC TTGCACTATT TGTACTCCTT TGACTTCCAC TAGCAATAGT ATCAGCAGCG GTAGGAGTAG
TAGTTTGTGA TTCTCCATTA TTAGTAGTAC TAGTACTTGC ACTATTTGTA CTCCTTTGAC TTCCACTAGC AATAGTATCA
GCAGCGGTAG GAGTAGTAGT TTGTGATTCT CCATTATTAG TAGTACTAGT ACTTGCACTA TTTGTACTAC TTTGACTTCC
ACTAGCAATA GTATCAGCAT TTGGAGCATT TGCACCTACA CTAGTAGTAT TAGAACCTTC ATTTGCCATA CTTCTCCTTA
TACTCATATT ATAAGCATTG TTTATGAATG TGTTGCTATA TTTACTTTC
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Date: 19 JUN 2016 **Signature:**

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