

Plasmodium falciparum, Strain Dd2

Catalog No. MRA-156

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain Dd2 is a clone derived from W2-MEF, which was selected from clone W2-MCII after 6 months of continuous cultivation in the presence of mefloquine. W2-MCII was derived from clone 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 is reported to be resistant to chloroquine, pyrimethamine and mefloquine. MRA-156 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 10 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.

Lot: 63999674

Manufacturing Date: 09FEB2016

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green [®] drug sensitivity assay ²		
Chloroquine	Report results	92.3 ± 2.1 nM
Artemisinin	Report results	17.2 ± 0.4 nM
Quinine	Report results	228.7 ± 10.5 nM
Cycloguanil	Report results	1145 ± 158.7 nM
Pyrimethamine	Report results	21230 ± 1958.1 nM
Sulfadoxine	Report results	439500 ± 30383.7 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 base pairs)	≥ 95% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: AASM01000018.1)	100% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: AASM01000018.1) (Figure 1)
Functional Activity by PCR Amplification¹ MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (10 days post-infection) ³		
Ring-stage parasitemia	Report results	2.18%
Total parasitemia	≥ 2%	3.97%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	2.36%
Total parasitemia	≥ 1%	3.65%
Viability (post-freeze; 4 days post-infection)¹	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)¹		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth

TEST	SPECIFICATIONS	RESULTS
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 Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vial, post-freeze material

²A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ values of an active (greater than 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>.]

³Testing completed on bulk material prior to vialing and freezing

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-156 MSP2 Sequence

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GTTACCTTTA ATATTAAAAA TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AATATGAGTA TAAGGAGAAG
TATGGCAAAAT GAAGGTTCTA ATACTACTAG TGTAGGTGCA AATGCTCCAA ATGCTGATAC TATTGCTAGT GGAAGTCAAAA
GTAGTACAAA TAGTGCAAGT ACTAGTACTA CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC TGATACTATT
GCTAGTGGA GTCAAAGGAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA CTACTCCTAC
CGCTGCTGAT ACTATTGCTA GTGGAAGTCA AAGGAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC
AACTACTAC TCCTACCGCT GCTGATACCC CTACTGCTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA
AGTTCAAGTT CTGGCAATGC ACCAAATAAA ACAGACGGTA AAGGAGAAGA GAGTGAAAAA CAAAATGAAT TAAATGAATC
AACTGAAGAA GGACCCAAAG CTCCACAAGA ACCTCAAACG GCAGAAAATG AAAATCCTGC TGCACCAGAG AATAAAGGTA
CAGGACAACA TGGACATATG CATGGTTCTA GAAATAATCA TCCACAAAAT ACTTCTGATA GTCAAAAAGA ATGTACCGAT
GGTAACAAAG AAAACTGTGG AGCAGCAACA TCCCTCTTAA ATAACTCTAG TAATATTGCT TCAATAAATA AATTTGTT
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