b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-156

Plasmodium falciparum, Strain Dd2

Catalog No. MRA-156

This reagent is the tangible property of the U.S. Government.

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% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 g per L D-glucose, 0.005 µg per mL hypoxanthine and 2.5 µg per 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 for 8 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: 70040128 **Manufacturing Date: 18NOV2020**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	31.0 ± 1.4 nM
Artemisinin	Report results	9.6 ± 0.2 nM
Quinine	Report results	89.1 ± 4.1 nM
Cycloguanil	Report results	1463 ± 287 nM
Pyrimethamine	Report results	17910 ± 1652 nM
Sulfadoxine	Report results	368700 ± 25558 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 870 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)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 850 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (8 days post-infection)[3]		
Ring-stage parasitemia	Report results	3.80%
Total parasitemia	≥ 2%	5.06%
Post-freeze (3 days post-infection)[1]		
Ring-stage parasitemia	Report results	2.82%
Total parasitemia	≥ 1%	4.64%
Viability (post-freeze; 3 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
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

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TEST	SPECIFICATIONS	RESULTS
Mycoplasma Contamination[1] DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material
[2]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.]
[3]Testing completed on bulk material prior to vialing and freezing
[4]Atlas, 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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AAAACATTGT CTATTATAAA TTTCTTTATT TTTGTTACCT TTAATATTAA AAATGAAAGT AAATATAGCA ACACATTCAT
AAACAATGCT TATAATATGA GTATAAGGAG AAGTATGGCA AATGAAGGTT CTAATACTAC TAGTGTAGGT GCAAATGCTC
CAAATGCTGA TACTATTGCT AGTGGAAGTC AAAGTAGTAC AAATAGTGCA AGTACTAGTA CTACTAATAA TGGAGAATCA
CAAACTACTA CTCCTACCGC TGCTGATACT ATTGCTAGTG GAAGTCAAAG GAGTACAAAT AGTGCAAGTA CTAGTACTAC
TAATAATGGA GAATCACAAA CTACTACTCC TACCGCTGCT GATACTATTG CTAGTGGAAG TCAAAGGAGT ACAAATAGTG
CAAGTACTAG TACTACTAAT AATGGAGAAT CACAAACTAC TACTCCTACC GCTGCTGATA CCCCTACTGC TACAGAAAGT
AATTCACCTT CACCACCCAT CACTACTACA GAAAGTTCAA GTTCTGGCAA TGCACCAAAT AAAACAGACG GTAAAGGAGA
AGAGAGTGAA AAACAAAATG AATTAAATGA ATCAACTGAA GAAGGACCCA AAGCTCCACA AGAACCTCAA ACGGCAGAAA
ATGAAAATCC TGCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT ATGCATGGTT CTAGAAATAA TCATCCACAA
AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGAAAACTG TGGAGCAGCA ACATCCCTCT TAAATAACTC
TAGTAATATT GCTTCAATAA ATAAATTTGT TGTTTTAATT TCAGCAACAC TTGTTTTATC TTTTG
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/Heather Couch/
Heather Couch 17 MAR 2021
Program Manager or designee, ATCC Federal Solutions

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