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SUPPORTING INFECTIOUS DISEASE RESEARCH

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. MRA-150 was produced by cultivation of BEI Resources seed 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, 27 µg/mL hypoxanthine and 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 for 19 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70052290 **Manufacturing Date: 09MAY2022**

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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	32.5 ± 0.7 nM
Artemisinin	Report results	11.9 ± 0.3 nM
Quinine	Report results	89.1 ± 4.1 nM
Cycloguanil	Report results	1421 ± 230 nM
Pyrimethamine	Report results	24240 ± 1117 nM
Sulfadoxine	Report results	278900 ± 38654 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)	99.9% sequence identity to *P. falciparum*, strain Dd2 (GenBank: AASM01000018.1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (19 days post-infection)[4]		
Ring-stage parasitemia	Report results	3.77%
Total parasitemia	≥ 2%	6.96%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	4.80%
Total parasitemia	≥ 1%	5.77%
Viability (2 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

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]
[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-150 MSP2 Sequence

AAAACATTGT CTATTATAAA TTTCTTTATT TTTGTTACCC 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 AATAAATTTG TTGTTTTAAT TTCAGCAACA CTTGTTTTAT CTTTTG

/Sonia Bjorum Brower/
Sonia Bjorum Brower 30 SEP 2022
Technical Manager or designee, ATCC Federal Solutions

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898