

**Plasmodium falciparum, Strain Dd2**

**Catalog No. MRA-150**

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 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<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) 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**

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: [Contact@BEIResources.org](mailto:Contact@BEIResources.org). We try to respond to feedback within 24 hours.

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>1</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)<sup>1</sup></b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR Green I <sup>®</sup> drug sensitivity assay <sup>2</sup>		
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
<b>Genotypic Analysis<sup>1</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 870 base pairs)	≥ 99% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: AASM01000018.1)	99.9% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: AASM01000018.1)
<b>Level of Parasitemia by Giemsa Stain Microscopy</b>		
Pre-freeze (19 days post-infection) <sup>4</sup>		
Ring-stage parasitemia	Report results	3.77%
Total parasitemia	≥ 2%	6.96%
Post-freeze (2 days post-infection) <sup>1</sup>		
Ring-stage parasitemia	Report results	4.80%
Total parasitemia	≥ 1%	5.77%
<b>Viability (2 days post-infection)<sup>1</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)<sup>1</sup></b>		
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup>	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

TEST	SPECIFICATIONS	RESULTS
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
<b>Mycoplasma Contamination<sup>1</sup></b> DNA detection by PCR	None detected	None detected

<sup>1</sup>Testing completed on vial, post-freeze material

<sup>2</sup>A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC<sub>50</sub> 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](http://www.beiresources.org).]

<sup>3</sup>Testing completed on bulk material prior to vialing and freezing

<sup>4</sup>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 CTACAGAAAAG
TAATTCACCT TCACCACCCA TCACTACTAC AGAAAAGTTCA AGTTCTGGCA ATGCACCAAA TAAAACAGAC GGTAAGGAG
AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA AGAAGGACCC AAAGCTCCAC AAGAACCCTCA AACGGCAGAA
AATGAAAATC CTGCTGCACC AGAGAATAAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA
AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAAC GTGGAGCAGC AACATCCCTC TTAATAACT
CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAAT TTCAGCAACA CTTGTTTTAT CTTTTG
    
```

/Sonia Bjorum Brower/  
Sonia Bjorum Brower

30 SEP 2022

Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

