

***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 MR-MRA-150 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, 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<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia daily 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: 70033211**

**Manufacturing Date: 25FEB2020**

| TEST  | SPECIFICATIONS   | RESULTS   |
|---|--|---|
| <b>Identification by Giemsa Stain Microscopy</b> <sup>1</sup>   | Blood-stage parasites present  | Blood-stage parasites present   |
| <b>Antimalarial Susceptibility Profile (<i>in vitro</i>)</b> <sup>1</sup><br>Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR green I <sup>®</sup> drug sensitivity assay <sup>2</sup><br>Chloroquine<br>Artemisinin<br>Quinine<br>Cycloguanil<br>Pyrimethamine<br>Sulfadoxine | Report results<br>Report results<br>Report results<br>Report results<br>Report results<br>Report results | 28.6 ± 0.7 nM<br>6.3 ± 0.1 nM<br>88.2 ± 4.1 nM<br>1462 ± 339.6 nM<br>29440 ± 1356 nM<br>317900 ± 29321 nM |
| <b>Genotypic Analysis</b> <sup>3</sup><br>Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 830 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) (Figure 1)         |
| <b>Functional Activity by PCR Amplification</b> <sup>3</sup><br>MSP2 PCR amplicon analysis  | ~ 600-900 base pair amplicon   | ~ 900 base pair amplicon  |
| <b>Level of Parasitemia by Giemsa Stain Microscopy</b><br>Pre-freeze (19 days post-infection) <sup>3</sup><br>Ring-stage parasitemia<br>Total parasitemia<br>Post-freeze (4 days post-infection) <sup>1</sup><br>Ring-stage parasitemia<br>Total parasitemia                                    | Report results<br>≥ 2%<br>Report results<br>≥ 1%   | 2.93%<br>5.21%<br>3.30%<br>4.63%  |
| <b>Viability (post-freeze; 4 days post-infection)</b> <sup>1</sup>  | Growth in infected red blood cells   | Growth in infected red blood cells  |
| <b>Sterility (21-day incubation)</b> <sup>1</sup><br>Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup><br>Trypticase soy broth, 37°C and 26°C, aerobic  | No growth<br>No growth   | No growth<br>No growth  |

| TEST  | SPECIFICATIONS | RESULTS       |
|---|----------------|---------------|
| 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     |
| <b>Mycoplasma Contamination<sup>1</sup></b><br>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<sup>®</sup> 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<sup>®</sup>-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>.

<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**

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TGTTACCTTT AATATTAAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAATGCTT ATAATATGAG TATAAGGAGA
AGTATGGCAA ATGAAGGTTT TAATACTACT AGTGTAGGTG CAAATGCTCC AAATGCTGAT ACTATTGCTA GTGGAAGTCA
AAGTAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACACTACT TCCTACCGCT GCTGATACTA
TTGCTAGTGG AAGTCAAAGG AGTACAAATA GTGCAAGTAC TAGTACTACT AATAATGGAG AATCACAAAC TACTACTCCT
ACCGCTGCTG ATACTATTGC TAGTGGAAAGT CAAAGGAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAACTACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAGTA ATTACCTTC ACCACCCATC ACTACTACAG
AAAGTTCAAG TTCTGGCAAT GCACCAAATA AAACAGACGG TAAAGGAGAA GAGAGTGAAA AACAAAATGA ATTAAATGAA
TCAACTGAAG AAGGACCCAA AGCTCCACAA GAACCTCAAA CGGCAGAAAA TGAAAATCCT GCTGCACCAG AGAATAAAGG
TACAGGACAA CATGGACATA TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG
ATGGTAACAA AGAAAACGTG GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA TAAATTTGTT
GTTTTAATTT CAGCAACACT TGTTTTATCT TTT
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29 APR 2020

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