

# Certificate of Analysis for MRA-1001

### Plasmodium falciparum, Strain 3D7 (GL Clone)

### Catalog No. MRA-1001

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

### **Product Description:**

The GL clone of 3D7 stock parasites was amplified in a volunteer patient "L", who participated in a clinical trial in August 1995 at Walter Reed Army Institute of Research (WRAIR), USA. *P. falciparum*, strain 3D7 (GL Clone) (available as BEI Resources MRA-102) was cloned from the NF54 strain (available as BEI Resources MRA-1000) by limiting dilution. The parent NF54 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands. MRA-1001 lot 70058154 was produced by cultivation of BEI Resources seed lot 58606906 in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27  $\mu$ g/mL hypoxanthine and 5  $\mu$ g/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia for 35 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: 70058154 Manufacturing Date: 22FEB2023

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy <sup>1</sup>	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (in vitro) <sup>1</sup> Half-maximal Inhibitory Concentration (IC50) by SYBR Green I® drug sensitivity assay <sup>2</sup>		
Chloroquine	Report results	8.6 ± 0.2 nM
Artemisinin	Report results	11.6 ± 0.3 nM
Quinine	Report results	70.3 ± 3.2 nM
Cycloguanil	Report results	13.0 ± 0.6 nM
Pyrimethamine	Report results	34.0 ± 3.9 nM
Sulfadoxine	Report results	278000 ± 51500 nM
Genotypic Analysis <sup>1</sup>		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 base pairs)	Consistent with P. falciparum	Consistent with <i>P. falciparum</i> (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (35 days post-infection) <sup>3</sup>		
Ring-stage parasitemia	Report results	2.57%
Total parasitemia	≥ 2%	4.37%
Post-freeze (2 days post-infection) <sup>1</sup>		
Ring-stage parasitemia	Report results	5.98%
Total parasitemia	≥ 1%	6.59%
Viability (2 days post-infection) <sup>1</sup>	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation) <sup>1</sup>		
Harpo's HTYE broth, 37°C and 26°C, aerobic⁴	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 <sup>1</sup> DNA detection by PCR	None detected	None detected

<sup>&</sup>lt;sup>1</sup>Testing completed on vialed, post-freeze material

#### Figure 1: MRA-1001 MSP2 Sequence

/Sonia Bjorum Brower/ Sonia Bjorum Brower

22 MAY 2023

Technical Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]

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

<sup>&</sup>lt;sup>4</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.