

Certificate of Analysis for MRA-151

Plasmodium falciparum, Strain 3D7A

Catalog No. MRA-151

Product Description:

Plasmodium falciparum (*P. falciparum*), strain 3D7A is a subclone of the 3D7 strain. *P. falciparum*, strain 3D7 (available as BEI Resources MRA-102) was cloned from the NF54 strain by limiting dilution. The parent NF54 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands. MRA-151 lot 70049010 was produced by cultivation of BEI Resources seed lot 59531004 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 μg/mL hypoxanthine and 5 μg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N_2 , 5% CO_2 , 5% O_2) and monitored for parasitemia for 12 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: 70049010 Manufacturing Date: 20DEC2021

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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	6.9 ± 0.3 nM
Artemisinin	Report results	12.5 ± 0.9 nM
Quinine	Report results	58.5 ± 5.4 nM
Cycloguanil	Report results	23.7 ± 1.6 nM
Pyrimethamine	Report results	86.8 ± 10.0 nM
Sulfadoxine	Report results	278200 ± 32100 nM
Genotypic Analysis ¹		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	Consistent with P. falciparum	Consistent with <i>P. falciparum</i> (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (12 days post-infection) ³		
Ring-stage parasitemia	Report results	2.7%
Total parasitemia	≥ 2%	4.6%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	0.6%
Total parasitemia	≥ 1%	2.4%
Viability (4 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, 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
Mycoplasma Contamination ¹		
DNA detection by PCR	None detected	None detected

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¹Testing completed on vialed, post-freeze material

²A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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.]

³Testing completed on bulk material prior to vialing and freezing

Figure 1: MRA-151 MSP2 Gene Sequence

/Sonia Bjorum Brower/ Sonia Bjorum Brower

06 JUN 2023

Technical Manager or designee, ATCC Federal Solutions

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⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.