

Certificate of Analysis for MRA-845

Plasmodium falciparum, Strain 3D7attB

Catalog No. MRA-845

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

Product Description:

Plasmodium falciparum (*P. falciparum*), strain 3D7^{attB} was generated by the integration of the acceptor *attB* site, recognized by the mycobacteriophage Bxb1 integrase during site-specific integration into the nonessential glutaredoxin-like *cg6* gene located on chromosome 7 of *P. falciparum*, strain 3D7. MRA-845 was produced by cultivation of the BEI Resources seed lot 58319584 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_2 , 5% CO_2 , 5% O_2) and monitored for parasitemia every 1 to 3 days for 42 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: 70062153 Manufacturing Date: 21SEP2023

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy ¹	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis ¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (730 base pairs)	≥ 99% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1)	100% sequence identity to P. falciparum, strain 3D7 (GenBank: LN999943.1) (Figure 1)
Antimalarial Susceptibility Profile (in vitro)1		
Half-maximal Inhibitory Concentration (IC50) by SYBR Green I® drug sensitivity assay ²		
Chloroquine	Report results	7.3 ± 0.5 nM
Artemisinin	Report results	22 ± 0.5 nM
Quinine	Report results	97.4 ± 6.7 nM
Cycloguanil	Report results	628.8 ± 101.8 nM
Pyrimethamine	Report results	38620 ± 1779.1 nM
Sulfadoxine	Report results	319100 ± 36819 nM
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (42 days post-infection) ³		
Ring-stage parasitemia	Report results	2.4%
Total parasitemia	≥ 2%	3.8%
Post-freeze (2 days post-infection) ¹		
Ring-stage parasitemia	Report results	2.9%
Total parasitemia	≥ 1%	3.7%
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, 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

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TEST	SPECIFICATIONS	RESULTS
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

¹Testing completed on vialed, post-freeze material.

Figure 1: MRA-845 MSP2 Sequence

/Sonia Bjorum Brower/ Sonia Bjorum Brower

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Technical Manager or designee, ATCC Federal Solutions

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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.

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