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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Plasmodium falciparum, Strain FCB

## Catalog No. MRA-309

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

## **Product Description:**

*Plasmodium falciparum (P. falciparum),* strain FCB originated in Southeast Asia and has shown resistance to chloroquine. MRA-309 lot 70058156 was produced by cultivation of BEI Resources seed lot 63901355 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 10 days. Every 1 to 2 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

# Lot: 70058156

# Manufacturing Date: 02FEB2023

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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 <sup>®</sup> drug sensitivity assay <sup>2</sup>		
Chloroquine	Report results	50.2 ± 1.2 nM
Artemisinin	Report results	19.8 ± 0.5 nM
Quinine	Report results	234.8 ± 5.4 nM
Cycloguanil	Report results	619.4 ± 143.9 nM
Pyrimethamine	Report results	30.2 ± 0.7 nM
Sulfadoxine	Report results	439300 ± 30370 nM
Genotypic Analysis <sup>1</sup>		
Sequencing of Merozoite Surface Protein 2 (MSP2)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i>
gene (~ 640 base pairs)		(Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (10 days post-infection) <sup>3</sup>		
Ring-stage parasitemia	Report results	6.2%
Total parasitemia	≥ 2%	7.8%
Post-freeze (3 days post-infection) <sup>1</sup>		
Ring-stage parasitemia	Report results	5.7%
Total parasitemia	≥ 1%	6.1%
Viability (3 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, 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
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination <sup>1</sup>		
DNA detection by PCR	None detected	None detected

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### <sup>1</sup>Testing completed on vialed, 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®-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>3</sup>Testing completed on bulk material prior to vialing and freezing

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

### Figure 1: MRA-309 MSP2 Sequence

TATTATAAATTTCTTTATTTTTGTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACATTCATAAACAATGCTTATAATATGAGTATAAGG TTCTGGTGATGGTAATGGTGCTAATCCTGGTGCAGATGCTGAGAGAAGTCCAAGTACTCCCGCTACTACCACAACTACCACAACTACTAATGATGCA GAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAATAATGCCGAAACAAATCAAGCAAATAAAGAAACTCAAAATAACTCAAATGYTCAAC AAGACTCTCAAACTAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCTACTGCACAACCTGAACAAGCTGAAAAATTCTGCTCCAAC AGCCGAACAAACTGAATCCCCCCGAATTACAATCTGCACCAGAGAATAAAGGTACAGG

/Sonia Bjorum Brower/ Sonia Bjorum Brower

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