

***Plasmodium falciparum*, Strain FCR-3/Gambia (Subline F-86)**

**Catalog No. MRA-731**

**Product Description:**

*Plasmodium falciparum* (*P. falciparum*), strain FCR-3/Gambia (subline F-86) was isolated in 1976 from the blood of a human patient with malaria in Gambia, West Africa. *P. falciparum*, strain FCR-3/Gambia was selected for knobby (K+) trait and has shown resistance to chloroquine. MRA-731 was produced by cultivation of the BEI Resources seed lot 58319490 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 every 1 to 3 days for 8 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: 70049774**

**Manufacturing Date: 25JAN2022**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>1</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)<sup>1</sup></b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR green I <sup>®</sup> drug sensitivity assay <sup>2</sup>		
Chloroquine	Report results	67.8 ± 6.3 nM
Artemisinin	Report results	8.5 nM ± 0.6 nM
Quinine	Report results	297.7 nM ± 20.6 nM
Cycloguanil	Report results	902.2 nM ± 62.4 nM
Pyrimethamine	Report results	68.5 nM ± 3.20 nM
Sulfadoxine	Report results	279000 nM ± 51685 nM
<b>Genotypic Analysis<sup>1</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
<b>Level of Parasitemia by Giemsa Stain Microscopy</b> Pre-freeze (8 days post-infection) <sup>3</sup>		
Ring-stage parasitemia	Report results	3.85%
Total parasitemia	≥ 2%	5.77%
Post-freeze (4 days post-infection) <sup>1</sup>		
Ring-stage parasitemia	Report results	3.67%
Total parasitemia	≥ 1%	4.99%
<b>Viability (post-freeze; 4 days post-infection)<sup>1</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)<sup>1</sup></b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</sup> Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination<sup>1</sup></b> DNA detection by PCR	None detected	None detected

<sup>1</sup>Testing completed on vialled, 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-731 MSP2 Sequence**

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TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAAATGCTT ATAATATGAG TATAAGGAGA
AGTATGACAG AAAGTAATCC TCCTACTGGT GCTAGTGGA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC
TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA
GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTTCTGGTGA TGGTAATGGT GCTAATCCTG GTGCAGATGC TGAGAGAAGT
CCAAGTACTC CCGTACTAC CACAACTACC ACAACTACTA ATGATGCAGA AGCATCTACC AGTACCTCTT CAGAAAATCC
AAATCATAAT AATGCCGAAA CAAATCAAGC AAATAAAGAA ACTCAAAATA ACTCAAATGT TCAACAAGAC TCTCAAAC TA
AATCAAATGT TCCACCCACT CAAGATGCAG ACACTAAAAG TCCTACTGCA CAACCTGAAC AAGCTGAAAA TTCTGCTCCA
ACAGCCGAAC AAAGTGAATC CCCC GAATTA CAATCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG
TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA CAAAGAAAAC TGCGGTCAGC
AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATT
    
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/Sonia Bjorum Brower/  
**Sonia Bjorum Brower**

24 JAN 2024

Technical Manager or designee, ATCC Federal Solutions

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