

Plasmodium falciparum, Strain FCR-3/Gambia Clone D-4, Knobless

Catalog No. MRA-739

Product Description:

Plasmodium falciparum (*P. falciparum*), strain FCR-3/Gambia Clone D-4, Knobless was derived from isolate FCR-3/FMG (Gambia) after four years of continuous culture by W. Trager by microscopic selection. Strain FCR-3/FMG (Gambia) was isolated in 1976 from the blood of a human patient in The Gambia, West Africa. MRA-739 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 12 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: 70019946

Manufacturing Date: 30OCT2018

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ²		
Chloroquine	Report results	22.9 ± 1.6 nM
Artemisinin	Report results	16.4 ± 0.8 nM
Quinine	Report results	106.7 ± 7.4 nM
Cycloguanil	Report results	933.3 ± 64.5 nM
Pyrimethamine	Report results	96.3 ± 11.1 nM
Sulfadoxine	Report results	399100 ± 46050 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (594 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
Functional Activity by PCR Amplification¹ MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (12 days post-infection) ³		
Ring-stage parasitemia	Report results	5.80%
Total parasitemia	≥ 2%	9.15%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	5.38%
Total parasitemia	≥ 1%	7.37%
Viability (post-freeze; 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, aerobic ⁴	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

TEST	SPECIFICATIONS	RESULTS
Mycoplasma Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vial, 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 (greater than 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. Available at: <https://www.beiresources.org/Publications/MethodsInMalariaResearch.aspx>.]

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.

Figure 1: MRA-739 MSP2 Sequence

```
GACAGAAAGT AATCCTCCTA CTGGTGCTAG TGGCAGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTGGT AGTGCTGGTG
GTAGTGCTGG TGGTTTTAAA AAGAAAGTGC TGGTGGTAGT GCTGAATTTA TTTATTGAAG CAATATTACT AGAGTTATTT
AAGAGGGATG TTGCTGCCCC ACAGTTTTCT TTGTTACCAT CGGTACATTC TTTTGGACTA TCAGAAGTAT TTTGTGGATG
ATTATTTCTA GAACCATGCA TATGTCCATG TTGTCTGTA CCTTTATTCT CTGGTGCAGA TTGTAATTCG GGGGATTTCAG
TTTGTTCGGC TGTTGGAGCA GAATTTTCAG CTGTTCAGG TTGTGCAGTA GGACTTTTAG TGTCTGCATC TTGAGTGGGT
GGAACATTTG ATTTAGTTTG AGAGTCTTGT TGAACATTTG AGTTATTTTG AGTTTCTTTA TTTGCTTGAT TTGTTTCGGC
ATTATTATGA TTTGGATTTT CTGAAGAGGT ACTGGTAGAT GCTTCTGCAT CATTAGTAGT TGTGGTAGTT GTGGTAGTAG
CGGGAGTACT TGGACTTCTC TCAGCATCTG CACC
```

/Heather Couch/
Heather Couch

18 MAY 2021

Program Manager or designee, ATCC Federal Solutions

ATCC[®], on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC[®]'s knowledge.

ATCC[®] is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

