

Plasmodium falciparum, Strain TM90C6A

Catalog No. MRA-204

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain TM90C6A was isolated from a patient in an atovaquone clinical trial in Thailand upon admission. The patient failed atovaquone treatment. MRA-204 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 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: 70042653

Manufacturing Date: 17MAR2021

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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	33.1 ± 1.5 nM
Artemisinin	Report results	10.5 ± 0.5 nM
Quinine	Report results	109.2 ± 15.1 nM
Cycloguanil	Report results	511.2 ± 118.8 nM
Pyrimethamine	Report results	16820 ± 774.9 nM
Sulfadoxine	Report results	310100 ± 26968.6 nM
Atovaquone		
24.4 nM to 25,000 nM	Report results	9125 ± 631 nM
0.122 nM to 125 nM	Report results	2.6 ± 0.2 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 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	~ 900 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (12 days post-infection) ³		
Ring-stage parasitemia	Report results	2.97%
Total parasitemia	≥ 2%	3.61%
Post-freeze (3 days post-infection) ¹		
Ring-stage parasitemia	Report results	1.27%
Total parasitemia	≥ 1%	1.90%
Viability (post-freeze; 3 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

TEST	SPECIFICATIONS	RESULTS
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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-204 MSP2 Sequence

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TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAACAAT
GCTTATAATA TGAGTATAAG GAGAAGTATG GAAGAAAGTA ATCCTTCTAC TGGTGCTGGT GGTAGTGGTA GTGCTGGTGG
TAGTGGTAGT GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA
GTGCTGGTGG TAGTGGTAGT GCTGGTTCTG GTGATGGTAA TGGTGCTAAT CCTGGTGCAG ATGCTGAGAG AAGTCCAAGT
ACTCCCGCTA CTACCACAAC TACCACAAC ACTAATGATG CAGAAGCATC TACCAGTACC TCTTCAGAAA ATCCAAATCA
TAATAATGCC GAAACAAATC CAAAAGGTAA AGGAGAAGTT CAAAAACCAA ATCAAGCAAA TAAAGAAACT CAAAATAACT
CAAATGTTCA ACAAGACTCT CAAACTAAAT CAAATGTTCC ACCCACTCAA GATGCAGACA CTAAAAGTCC TACTGCACAA
CCTGAACAAG CTGAAAATTC TGCTCCAACA GCCGAACAAA CTGAATCCCC CGAATTACAA TCTGCACCAG AGAATAAAGG
TACAGGACAA CATGGACATA TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG
ATGGTAACAA AGAAAAGTGT GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA TAAATT
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