

Certificate of Analysis for MRA-926

Plasmodium falciparum, Strain 7G8

Catalog No. MRA-926

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

Product Description:

Plasmodium falciparum (*P. falciparum*), strain 7G8 was cloned from the IMTM22 strain by limiting dilution. The original IMTM22 strain was isolated from a 12-year-old male near Manaus, Brazil in 1980. MRA-926 was produced by cultivation of BEI Resources MR-MRA-926 lot 58422570 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 2.5μ g/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 daily 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: 63079297 Manufacturing Date: 11NOV2014

TEST	SPECIFICATIONS	RESULTS		
Identification by Giemsa Stain Microscopy ¹	Blood-stage parasites present	Blood-stage parasites present		
Antimalarial Susceptibility Profile (in vitro) ¹				
Half-maximal Inhibitory Concentration (IC50) by SYBR				
green I [®] drug sensitivity assay ²		10.4 . 0.4 . 14		
Chloroquine	Report results	46.1 ± 6.4 nM		
Artemisinin	Report results	3.9 ± 0.5 nM 63.9 ± 10.3 nM 614.8 ± 42.5 nM 44980 ± 4148.3 nM 227600 ± 170025 nM		
Quinine	Report results			
Cycloguanil	Report results			
Pyrimethamine	Report results			
Sulfadoxine	Report results			
Genotypic Analysis ¹				
Sequencing of Merozoite Surface Protein 2 (MSP2)	≥ 99% sequence identity to	100% sequence identity to		
gene (~ 800 base pairs)	P. falciparum, strain 7G8	P. falciparum, strain 7G8		
	(GenBank: ABGZ02000545)	(GenBank: ABGZ02000545) (Figure 1)		
MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 900 base pair amplicon		
Level of Parasitemia by Giemsa Stain Microscopy				
Pre-freeze (8 days post-infection) ³				
Ring-stage parasitemia	Report results	5.42%		
Total parasitemia	≥ 2%	7.50%		
Post-freeze (4 days post-infection) ¹				
Ring-stage parasitemia	Report results	5.23%		
Total parasitemia	≥ 1%	8.93%		
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		
Mycoplasma Contamination ¹		_		
DNA detection by PCR	None detected	None detected		

¹Testing completed on vialed, post-freeze material

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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. Available at: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx.]

Figure 1: MRA-926 MSP2 Sequence

AATATGGCAA	AAGATAAAAC	AAGTGTTGCT	GAAATTAAAA	CAACAAATTT	ATTTATTGAA	GCAATATTAC	TAGAGTTACT
TAAGAGGGAT	GGTGCTGCTC	CACAGTTTTC	TTTGTTACCA	TCGGTACATT	CTTTTTGACT	ATCAGAAGTA	TTTTGTGGAT
GATTATTTCT	AGAACCATGC	ATATGTCCAT	GTTGTCCTGT	ACCTTTATTC	TCTGGTGCAG	ATTGTAATTC	GGGGGATTCA
GTTTGTTCGG	CTATTGGAGC	AGAATTTTCA	GCTTGTTCAG	GTTGTGCAGT	AGGACTTTTA	GTGTCTGCAT	CTTGAGTGGG
TGGAACATTT	GATTTAGTTT	GAGAGTCTTG	TTGAACATTT	GAGTTATTTT	GAGTTTCTTT	ATTTGCTTGA	TTTGGTTTTT
GAACTTCTCC	TTTACCTTTT	GGATTTGTTT	CGGCATTATT	ATGATTTGGA	TTTTCTGAAG	AGGTACTGGT	AGATGCTTCT
GCATCATTAG	TAGTTGTGGT	AGTTGTGGTA	GTAGCGGGAG	TACTTGGACT	TCTCTCAGCA	TCTGCACCAG	GATTAGCACC
ATTACCATCA	CCAGAACCAG	CACTACCACT	ACCACCAGCA	CTACCACTAC	CACCAGCACT	ACCACTACCA	CCAGCACTAC
CACTACCACC	AGCACTACCA	CTACCACCAG	CACCAGTAGA	AGGATTACTT	TCTGCCATAC	TTCTCCTTAT	ACTCATATTA
TAAGCATTGT	TTATGAATGT	GTTGCTATAT	TTACTTTCAT	TTTTAATATT	AAAGGTAACA	AAAATAAAGA	AATTTATAAT
AGACAATGTT	TTAA						

/Heather Couch/

Heather Couch 23 JUL 2020

Program Manager or designee, ATCC Federal Solutions

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³Testing completed on bulk material prior to vialing and freezing

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