

***Plasmodium falciparum*, Strain FCR-8/West African**

Catalog No. MRA-732

Product Description:

Plasmodium falciparum (*P. falciparum*), strain FCR-8/West African was originally isolated from the blood of a human patient collected in 1978 in West Africa. MRA-732 was derived from ATCC® 50028™, which was deposited to ATCC® by W. Trager. *P. falciparum*, strain FCR-8/West African was identified as sensitive to chloroquine. MRA-732 was produced by cultivation of seed material 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia 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: 70017001

Manufacturing Date: 24AUG2018

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	7.4 ± 0.5 nM
Artemisinin	Report results	9.6 ± 0.7 nM
Quinine	Report results	23.8 ± 1.6 nM
Cycloguanil	Report results	136.9 ± 15.8 nM
Pyrimethamine	Report results	63.4 ± 7.3 nM
Sulfadoxine	Report results	428400 ± 29616 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 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	~ 600-900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (8 days post-infection) ³		
Ring-stage parasitemia	Report results	3.39%
Total parasitemia	≥ 2%	4.71%
Post-freeze (3 days post-infection) ¹		
Ring-stage parasitemia	Report results	1.24%
Total parasitemia	≥ 1%	4.23%
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
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 (> 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-732 MSP2 Sequence

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CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAAACAAT GCTTTATAAT ATGAGTATAA GGAGAAGTAT
GAAAGAAAGT AAGCCTCCTA CTGGTGCTAG TGGTAGTGCT GGTCTGGT CTGGTGCTGT TGCTAGTGCT GGTAATGGTG
CTAATCCTGG TGCAGATGCT GAGAGAAGTC CAAGTACTCC CGTACTCCC GCTACTCCC CTACTCCCGC TACTCCCGCT
ACTCCCGCTA CTCCCGCTAC TACCACAACCT ACCACAACCTA CCACAACCTAC CACAACCTACT AATGATGCAG AAGCATCTAC
CAGTACCTCT TCAGAAAATC CAAATCATAA TAATGCCAAA ACAAATCCAA AAGGTAATGG AGGAGTTCAA GAACCAAATA
AAGCAAATAC AGAAACTCAA AATAACTCAA ATGTTCAACA AGACTCTCAA ACTAAATCAA ATGTTCCACC CACTCAAGAT
GCAGACACTA AAAGTCCTAC TGCACAACCT GAACAAGCTG AAAATTCTGC TCCAACAGCC GAACAACTG AATCCCCCGA
ATTACAATCT GCACCAGAGA ATAAAGGTAC AGGACAACAT GGACATATGC ATGGTTCTAG AAATAATCAT CCACAAAATA
CTTCTGATAG TCAAAAAGAA TGTACCGATG GTAACAAAGA AACTGTGGA GCAGCAACAT CCCTCTTAA TAACTCTAGT
AATATTGCTT CAATAAATAA ATTTGTTG
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10 DEC 2020

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