

Plasmodium falciparum, Strain LA476-1

Catalog No. MRA-1330

Product Description:

Plasmodium falciparum (*P. falciparum*), strain LA476-1 is a clone of *P. falciparum*, strain LA476, which was isolated in 2008 from a patient in Malawi. Strain LA476-1 is the progenitor of two deletion mutants, strains LA476-1 Δ *hrp2* (MRA-1331) and LA476-1 Δ *hrp2*/ Δ *hrp3*, generated by the deletion of *P. falciparum* histidine rich protein 2 (*hrp2*) and 3 (*hrp3*) genes, located outside of the telomeric regions of chromosomes 8 and 13.2, respectively, using CRISPR/Cas9-technology. MRA-1330 was produced by cultivation of deposited material in fresh human erythrocytes suspended in RPMI 1640 medium adjusted to contain 10% (v/v) heat-inactivated human serum (Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 μ g/mL hypoxanthine and 5 μ g/mL gentamicin. The culture was propagated in human Type O erythrocytes at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 14 days. Every 1 to 4 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise as needed to maintain 1% to 3% hematocrit. The culture was harvested when the total parasitemia reached \geq 2% to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70064017

Manufacturing Date: 09OCT2023

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy ¹	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis ¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 640 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
Confirmation of Genes by PCR Amplification ^{1,2} <i>hrp2</i> <i>hrp3</i>	~ 300 base pair amplicon ~ 300 base pair amplicon	~ 300 base pair amplicon ~ 300 base pair amplicon
Antimalarial Susceptibility Profile (in vitro) ¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR Green I® drug sensitivity assay ³ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	5.4 \pm 0.4 nM 19.1 \pm 1.8 nM 100.2 \pm 11.6 nM 940.4 \pm 65 nM 33740 \pm 2333 nM 254500 \pm 23474 nM
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (14 days post-infection) ⁴ Ring-stage parasitemia Total parasitemia Post-freeze (2 days post-infection) ¹ Ring-stage parasitemia Total parasitemia	Report results \geq 2% Report results \geq 1%	8.2% 10.9% 1.1% 1.5%
Viability (2 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 ⁵ Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic	No growth No growth No growth	No growth No growth No growth

TEST	SPECIFICATIONS	RESULTS
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.

²Primer sequences and conditions for PCR are available upon request.

³A 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: Moll, K., et al. (Eds.), *Methods in Malaria Research*. 6th ed. EVIMalaR, 2013. 122-129. *Methods in Malaria Research Sixth Edition* is available on the [BEI Resources website](http://www.beiresources.org).]

⁴Testing completed on bulk material prior to vialing and freezing.

⁵Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1330 MSP2 Sequence

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AAATGAAGGTTCTAATACTAATAAGTGTAGGTGCAGATGCTCCAAAAGCTGATACTATTGCTAGTGGAAAGTCAAAGTAGTACAAATAG
TGCAAGTACTAGTACTACTAATAATGGAGAATCACAACTACTACTCCTACCGCTGCTGATACCCCTACTGCTACAGAAAGTAATTC
ACCTTCACCACCCATCACTACTACAAAAAGTAATTCACCTTCACCACCCATCACTACTACAAAAAGTAATTCACCTTCACCACCCAT
CACTACTACAAAAAGTAATTCACCTTCACCACCCATCACTACTACAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAACAGACGG
TAAAGGAGAAGAGAGTGA AAAACAAAATGAATTAATGAATCAACTGAAGAAGGACCCAAAGCTCCACAAGAACCTCAAACGGCAGA
AAATGAAAATCCTGCTGCACCAGAGAATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATAC
TTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAAGAAAAGTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATATTGC
TTCAATAAATAAATTTGTTGTTTTAATT
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25 MAR 2024

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