Certificate of Analysis for MRA-1332

Plasmodium falciparum, Strain LA476-1Δ*hrp*2/Δ*hrp*3

Catalog No. MRA-1332

Product Description:

Plasmodium falciparum (P. falciparum), strain LA476-1Δ*hrp*2/Δ*hrp*3 is deletion mutant derived from the progenitor strain LA476-1 (MRA-1330). Strain LA476-1 is a clone of *P. falciparum, strain* LA476, which was originally isolated in 2008 from a patient in Malawi. Strain LA476-1Δ*hrp*2/Δ*hrp*3 was generated by the deletion of the *P. falciparum* histidine-rich protein 2 (*hrp*2) and 3 (*hrp*3) genes, located outside of the telomeric region of chromosomes 8 and 13.2, respectively, using CRISPR-Cas9 technology. MRA-1332 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 µg/mL hypoxanthine and 5 µg/mL gentamicin. The culture was propagated in human Type O erythrocytes at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia for 22 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 ≥ 2% to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70064019 **Manufacturing Date: 17OCT2023**

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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 (~ 510 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Confirmation of Gene Deletion by PCR Amplification[1,2]		
*hrp*2 (MRA-1332)	No amplicon	No amplicon
*hrp*2 (MRA-1330; positive control)	~ 300 base pair amplicon	~ 300 base pair amplicon
*hrp*3 (MRA-1332)	No amplicon	No amplicon
*hrp*3 (MRA-1330; positive control)	~ 300 base pair amplicon	~ 300 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (22 days post-infection)[3]		
Ring-stage parasitemia	Report results	5.0%
Total parasitemia	≥ 2%	6.1%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	2.4%
Total parasitemia	≥ 1%	3.2%
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[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

[1]Testing completed on vialed, post-freeze material.
[2]Primer sequences and conditions for PCR are available upon request.

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[3]Testing completed on bulk material prior to vialing and freezing.
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1332 MSP2 Sequence

AACTACTACTCCTACCGCTGCTGATACCCCTACTGCTACAGAAAGTAATTCACCTTCACCACCCATCACTACTACAAAAAGTAATTC
ACCTTCACCACCCATCACTACTACAAAAAGTAATTCACCTTCACCACCCATCACTACTACAAAAAGTAATTCACCTTCACCACCCAT
CACTACTACAGAAAGTTCAAGTTCTGGCAATGCACCAAATAAAACAGACGGTAAAGGAGAAGAGAGTGAAAAACAAAATGAATTAAA
TGAATCAACTGAAGAAGGACCCAAAGCTCCACAAGAACCTCAAACGGCAGAAAATGAAAATCCTGCTGCACCAGAGAATAAAGGTAC
AGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAA
AGAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATT

/Sonia Bjorum Brower/
Sonia Bjorum Brower 02 APR 2024
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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898