Certificate of Analysis for MRA-1324

Plasmodium falciparum, Strain NF54-*cg6-ULG8*-CBG99

Catalog No. MRA-1324

Product Description:

MRA-1324 is a *Plasmodium falciparum (P. falciparum)* parasite reporter strain produced by integrating the *ULG8*-CBG99 luciferase reporter cassette into the *cg6* gene locus of *P. falciparum* parasite line NF54^{attB}. MRA-1324 was produced by cultivation of deposited material in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled 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 incubated 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 14 days. Every 1 to 4 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70058847 **Manufacturing Date: 03MAR2023**

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity of Luciferase Gene[1,3]	Positive	Positive
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (14 days post-infection)[2]		
Ring-stage parasitemia	Report results	3%
Total parasitemia	≥ 2%	6%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	2.6%
Total parasitemia	≥ 1%	2.8%
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]Testing completed on bulk material prior to vialing and freezing
[3]Luciferase activity was determined using the Luciferase Assay System (Promega E1500). Parasites were lysed and incubated with luciferase assay reagent. Luciferase activity was measured using a luminometer with a bioluminescence emission spectra of ~ 540 nm.
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Figure 1: MRA-1324 MSP2 Sequence

ATGAAGGTAATTAAAACATTGTCTATTATAAATTTCTTTATTTTTGTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACA
TTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCAGAAAGTAAGCCTTCTACTGGTGCTGGTGGTAGTGCTGGTGGT
AGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTTCTGGTGATGGTAATGGTGCAGATGCTGAG
GGAAGTTCAAGTACTCCCGCTACTACCACAACTACCAAAACTACCACAACTACCACAACTACTAATGATGCAGAAGCATCTACCAGT
ACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAACAAATCCAAAAGGTAAAGGAGAAGTTCAAGAACCAAATCAAGCAAATAAA
GAAACTCAAAATAACTCAAATGTTCAACAAGACTCTCAAACTAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCT
ACTGCACAACCTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAATAAA
GGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGT
AACAAAGAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTCA
GCAACACTTGTTTTATCTTTTGCCATATTCATATAAA

/Sonia Bjorum Brower/
Sonia Bjorum Brower 14 MAR 2024
Technical Manager or designee, ATCC Federal Solutions

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