Certificate of Analysis for MRA-1217

Plasmodium falciparum, Strain NF54HT-GFP-luc

Catalog No. MRA-1217

Product Description:

Plasmodium falciparum (P. falciparum), strain NF54HT-GFP-luc is a recombinant clone produced in 2012 by single crossover integration of green fluorescent protein-luciferase (GFP-luc) in the NF54 (patient line E) strain (available as BEI Resources MRA-1000). The parent NF54 strain was isolated from a patient living in the Netherlands, who had never left the country. Strain NF54HT-GFP-luc expresses cytoplasmic GFP-luciferase in all life cycle stages. MRA-1217 was produced by cultivation of BEI Resources seed lot 62349333 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, 27 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia every 1 to 3 days for 13 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: 70064597 **Manufacturing Date: 21NOV2023**

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC50) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	9.6 ± 0.4 nM
Artemisinin	Report results	10.8 ± 2.0 nM
Quinine	Report results	67.9 ± 9.4 nM
Cycloguanil	Report results	278.8 ± 38.6 nM
Pyrimethamine	Report results	20350 ± 3770 nM
Sulfadoxine	Report results	504400 ± 46523 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	≥ 95% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292.1)	100% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292.1) (Figure 1)
Phenotypic Analysis[1]		
GFP expression	Positive	Positive
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (13 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.3%
Total parasitemia	≥ 2%	3.4%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	1.8%
Total parasitemia	≥ 1%	1.8%
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

BEI Resources
www.beiresources.org

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

Certificate of Analysis for MRA-1217

TEST	SPECIFICATIONS	RESULTS
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]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (greater than 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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]
[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-1217 MSP2 Sequence

GTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACATTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCA
GAAAGTAAGCCTTCTACTGGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGT
GGTAGTGCTGGTTCTGGTGATGGTAATGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCGCTACTACCACAACTACCAAAACTACC
ACAACTACCACAACTACTAATGATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAACAAATCCA
AAAGGTAAAGGAGAAGTTCAAGAACCAAATCAAGCAAATAAAGAAACTCAAAATAACTCAAATGTTCAACAAGACTCTCAAACTAAA
TCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCTACTGCACAACCTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAA
CAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCAT
CCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAGAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCT
AGTAATATTGCTTCAATAAATAAATTTG

/Sonia Bjorum Brower/
Sonia Bjorum Brower 06 NOV 2024
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.