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.

Lot[1]: 70014465 **Manufacturing Date: 09MAY2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2] Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[4] Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	 Report results Report results Report results Report results Report results Report results	 7.9 ± 0.5 nM 8.3 ± 0.4 nM 44.7 ± 2.1 nM 288.7 ± 67.1 nM 25590 ± 3547 nM 368700 ± 34007 nM
Genotypic Analysis[2] Sequencing of Merozoite Surface Protein 2 (MSP2) gene (765 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292)	100% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292) (Figure 1)
Functional Activity by PCR Amplification[2] MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Phenotypic Analysis GFP expression	Positive	Pending
Level of Parasitemia Pre-freeze[6,7] Ring-stage parasitemia Total parasitemia Post-freeze[2,8] Ring-stage parasitemia Total parasitemia	 Report results ≥ 2% Report results ≥ 1%	 2.65% 3.85% 0.39% 2.16%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2] Harpo's HTYE broth[10], 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	 No growth No growth No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination[2] DNA Detection by PCR	None detected	None detected

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[1]MRA-1217 was produced by cultivation of the MR-MRA-1217 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, 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 19 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.
[2]Testing completed on vialed post-freeze material.
[3]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 4 days.
[4]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} 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].
[5]Primer sequences and conditions for PCR are available upon request.
[6]Testing completed on bulk material prior to vialing and freezing.
[7]Parasitemia was determined after 19 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[10]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

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GTTACCTTTA ATATTAAAAA TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AATATGAGTA TAAGGAGAAG
TATGGCAGAA AGTAAGCCTT CTACTGGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG
GTGGTAGTGC TGGTGGTAGT GCTGGTTCTG GTGATGGTAA TGGTGCAGAT GCTGAGGGAA GTTCAAGTAC TCCCGCTACT
ACCACAACTA CCAAAACTAC CACAACTACC ACAACTACTA ATGATGCAGA AGCATCTACC AGTACCTCTT CAGAAAATCC
AAATCATAAA AATGCCGAAA CAAATCCAAA AGGTAAAGGA GAAGTTCAAG AACCAAATCA AGCAAATAAA GAAACTCAAA
ATAACTCAAA TGTTCAACAA GACTCTCAAA CTAAATCAAA TGTTCCACCC ACTCAAGATG CAGACACTAA AAGTCCTACT
GCACAACCTG AACAAGCTGA AAATTCTGCT CCAACAGCCG AACAAACTGA ATCCCCCGAA TTACAATCTG CACCAGAGAA
TAAAGGTACA GGACAACATG GACATATGCA TGGTTCTAGA AATAATCATC CACAAAATAC TTCTGATAGT CAAAAAGAAT
GTACCGATGG TAACAAAGAA AACTGTGGAG CAGCAACATC CCTCTTAAAT AACTCTAGTA ATATTGCTTC AATAAATAAA
TTTGTTGTTT TAATTTCAGC AACACTTGTT TTATCTTTTG CCATA
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/Heather Couch/
Heather Couch 18 DEC 2018
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