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]: 62349332 **Manufacturing Date: 17JUN2014**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	7.4 ± 0.3 nM
Artemisinin	Report results	4.5 ± 0.5 nM
Quinine	Report results	34.1 ± 3.1 nM
Cycloguanil	Report results	328 ± 156.7 nM
Pyrimethamine	Report results	39200 ± 4523 nM
Sulfadoxine	Report results	323600 ± 44849 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292)	100% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Phenotypic Analysis		
GFP expression[5]	Positive	Positive (Figure 2)
Level of Parasitemia		
Pre-freeze[6]		
Ring-stage parasitemia	Report results	9.80%
Total parasitemia	≥ 2%	12.0%
Post-freeze[7]		
Ring-stage parasitemia	Report results	6.48%
Total parasitemia	≥ 1%	7.32%
Viability (post-freeze)[8]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)		
Harpo's HTYE broth[9], 37°C and 26°C, aerobic	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose 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		
DNA Detection by PCR	None detected	None detected

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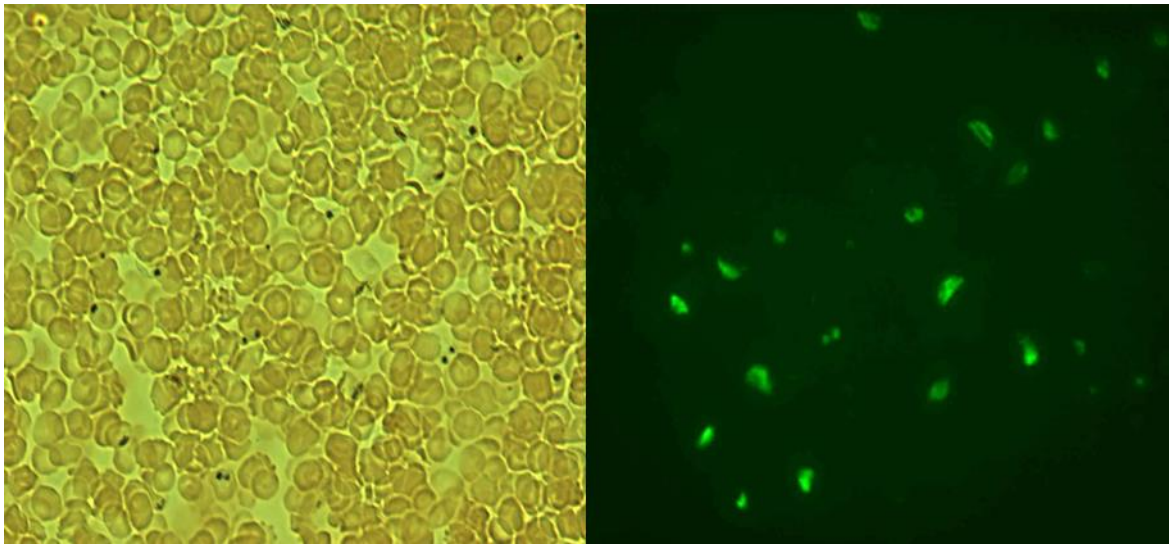
[1]MRA-1217 was produced by cultivation of the deposited material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 g/L D-glucose, 0.005 µg/mL hypoxanthine and 2.5 µg/mL gentamicin. The 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 4 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]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.
[3]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].
[4]Primer sequences and conditions for PCR are available upon request.
[5]GFP expression was monitored by fluorescence microscopy. Cytoplasmic fluorescence specific to parasites was observed.
[6]Pre-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.
[9]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

GAAAGTAAAT ATAGCAACAC ATTCATAAAC AATGCTTATA ATATGAGTAT AAGGAGAAGT ATGGCAGAAA GTAAGCCTTC
TACTGGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTGGT AGTGCTGGTG GTAGTGCTGG TGGTAGTGCT GGTGGTAGTG
CTGGTTCTGG TGATGGTAAT GGTGCAGATG CTGAGGGAAG TTCAAGTACT CCCGCTACTA CCACAACTAC CAAAACTACC
ACAACTACCA CAACTACTAA TGATGCAGAA GCATCTACCA GTACCTCTTC AGAAAATCCA AATCATAAAA ATGCCGAAAC
AAATCCAAAA GGTAAAGGAG AAGTTCAAGA ACCAAATCAA GCAAATAAAG AAACTCAAAA TAACTCAAAT GTTCAACAAG
ACTCTCAAAC TAAATCAAAT GTTCCACCCA CTCAAGATGC AGACACTAAA AGTCCTACTG CACAACCTGA ACAAGCTGAA
AATTCTGCTC CAACAGCCGA ACAAACTGAA TCCCCCGAAT TACAATCTGC ACCAGAGAAT AAAGGTACAG GACAACATGG
ACATATGCAT GGTTCTAGAA ATAATCATCC ACAAAATACT TCTGATAGTC AAAAAGAATG TACCGATGGT AACAAAGAAA
ACTGTGGAGC AGCAACATCC CTCTTAAATA ACTCTAGTAA TATTGCTTCA ATAAATAAAT TTGTTGTTTT AATTTCAGCA
ACACTTGTTT TATCTTTTGC CATATTCATA TAA

Figure 2: GFP Expression by MRA-1217



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Heather Couch 18 DEC 2018
Program Manager or designee, ATCC Federal Solutions

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