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SUPPORTING INFECTIOUS DISEASE RESEARCH

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 seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per 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 for 11 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: 70042651 **Manufacturing Date: 08MAR2021**

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	8.1 ± 0.6 nM
Artemisinin	Report results	7.1 ± 0.3 nM
Quinine	Report results	42.0 ± 3.9 nM
Cycloguanil	Report results	324 ± 29.9 nM
Pyrimethamine	Report results	49220 ± 2268 nM
Sulfadoxine	Report results	497700 ± 22928 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (805 base pairs)	≥ 95% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292)	99.9% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292) (Figure 1)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	~ 800 base pair amplicon
Phenotypic Analysis		
GFP expression	Positive	Positive
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (11 days post-infection)[3]		
Ring-stage parasitemia	Report results	3.15%
Total parasitemia	≥ 2%	5.68%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.71%
Total parasitemia	≥ 1%	2.85%
Viability (post-freeze; 4 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

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TEST	SPECIFICATIONS	RESULTS
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]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. Available at: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx.]
[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

CATTGTCTAT TATAAATTTC TTTATTTTTG TTACCTTTAA TATTAAAAAT 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 CATATTTCAT
ATAAA

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Heather Couch 21 APR 2021
Program Manager or designee, ATCC Federal Solutions

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