

Plasmodium falciparum, Strain NF54 (Patient Line E)

Catalog No. MRA-1000

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Product Description:

The E line of NF54 stock parasites was amplified in a volunteer patient "E," who participated in a clinical trial in 1995 at Walter Reed Army Institute of Research (WRAIR). The parent NF54 strain of *Plasmodium falciparum* (*P. falciparum*) was isolated from a patient living near an airport in the Netherlands, who had never left the country. MRA-1000 was produced by cultivation of BEI Resources seed lot 58607195 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 milliliter hypoxanthine and 2.5 micrograms per milliliter gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 9 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: 70041145

Manufacturing Date: 20JAN2021

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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	8.6 ± 0.6 nM
Artemisinin	Report results	9.1 ± 0.6 nM
Quinine	Report results	87.9 ± 4.0 nM
Cycloguanil	Report results	42.5 ± 2.9 nM
Pyrimethamine	Report results	53.7 ± 6.2 nM
Sulfadoxine	Report results	423000 ± 39015 nM
Genotypic Analysis ¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 base pairs)	≥ 95% sequence identity to <i>P. falciparum</i> , strain NF54 (GenBank: AMYQ01000292)	100% sequence identity to <i>P. falciparum</i> , 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
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (9 days post-infection) ³		
Ring-stage parasitemia	Report results	3.96%
Total parasitemia	≥ 2%	6.27%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	6.88%
Total parasitemia	≥ 1%	8.15%
Viability (post-freeze; 4 days post-infection) ¹	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (22-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

TEST	SPECIFICATIONS	RESULTS
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vial, post-freeze material

²A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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>.]

³Testing completed on bulk material prior to vialing and freezing

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1000 MSP2 Sequence

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ATTAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTAAATAT TAAAAATGAA AGTAAATATA GCAACACATT
CATAAACAAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAGAAAGTA AGCCTTCTAC TGGTGCTGGT GGTAGTGCTG
GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTTCTGGTGA TGGTAATGGT
GCAGATGCTG AGGGAAGTTC AAGTACTCCC GCTACTACCA CAACTACCAA AACTACCACA ACTACCACAA CTACTAATGA
TGCAGAAGCA TCTACCAGTA CCTCTCAGA AAATCCAAAT CATAAAAATG CCGAAACAAA TCCAAAAGGT AAAGGAGAAG
TTCAAGAACC AAATCAAGCA AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT CTCAAACTAA ATCAAATGTT
CCACCCACTC AAGATGCAGA CACTAAAAGT CCTACTGCAC AACCTGAACA AGCTGAAAAT TCTGCTCAA CAGCCGAACA
AACTGAATCC CCCGAATTAC AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA
ATCATCCACA AAATACTTCT GATAGTCAA AAGAATGTAC CGATGGTAAC AAAGAAAAC TGGGAGCAGC AACATCCCTC
TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATT
    
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17 SEP 2021

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