Certificate of Analysis for MRA-102

Plasmodium falciparum, Strain 3D7

Catalog No. MRA-102

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Product Description: *Plasmodium falciparum (P. falciparum)*, strain 3D7 was cloned from the NF54 strain by limiting dilution; it is reported as a pyrimethamine-sensitive strain. The parent NF54 strain was isolated from a patient from the Netherlands who had never left the country.

Lot[1]: 64202795 **Manufacturing Date: 14MAY2016**

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	Report results	8.4 ± 0.6 nM
Artemisinin	Report results	8.8 ± 0.4 nM
Quinine	Report results	92.7 ± 8.6 nM
Cycloguanil	Report results	8.0 ± 0.6 nM
Pyrimethamine	Report results	39.0 ± 2.7 nM
Sulfadoxine[5]	Report results	371800 ± 77602 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 820 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943.1)	99.7% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943.1) (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[6]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[7,8]		
Ring-stage parasitemia	Report results	3.05%
Total parasitemia	≥ 2%	4.83%
Post-freeze[2,9]		
Ring-stage parasitemia	Report results	1.09%
Total parasitemia	≥ 1%	2.84%
Viability[2,10]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[11], 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[2]		
DNA Detection by PCR	None detected	None detected

[1]MRA-102 was produced by cultivation of BEI Resources MR-MRA-102 lot 63085271 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,

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5% CO_2, 5% O_2) and monitored for parasitemia daily for 17 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 14 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]The high-dilution results are shown, as the low-dilution results were not interpretable for this lot.

[6]Primer sequences and conditions for PCR are available upon request.

[7]Testing completed on bulk material prior to vialing and freezing.

[8]Parasitemia was determined after 17 days post infection by microscopic counts of Giemsa-stained blood smears.

[9]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.

[10]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.

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

Figure 1: MRA-102 MSP2 Sequence

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AGGTAATTAA AACATTGTCT ATTATAAATT TCTTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA
CACATTCATA AACAATGCTT ATAATATGAG TATAAGGAGA AGTATGGCAG AAAGTAAGCC TTCTACTGGT GCTGGTGGTA
GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTTC TGGTGATGGT
AATGGTGCAG ATGCTGAGGG AAGTTCAAGT ACTCCCGCTA CTACCACAAC TACCAAAACT ACCACAACTA CCACAACTAC
TAATGATGCA GAAGCATCTA CCAGTACCTC TTCAGAAAAT CCAAATCATA AAAATGCCGA AACAAATCCA AAAGGTAAAG
GAGAAGTTCA AGAACCAAAT CAAGCAAATA AAGAAACTCA AAATAACTCA AATGTTCAAC AAGACTCTCA AACTAAATCA
AATGTTCCAC CCACTCAAGA TGCAGACACT AAAAGTCCTA CTGCACAACC TGAACAAGCT GAAAATTCTG CTCCAACAGC
CGAACAAACT GAATCCCCCG AATTACAATC TGCACCAGAG AATAAAGGTA CAGGACAACA TGGACATATG CATGGTTCTA
GAAATAATCA TCCACAAAAT ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG AAAACTGTGG AGCAGCAACA
TCCCTCTTAA ATAACTCTAG TAATATTGCT TCAATAAATA AATTTGTTGT TTTAATTTCA GCAAACACTT GTTTTATCTT
TTGCCATATT CATATA
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