Certificate of Analysis for MRA-102

Plasmodium falciparum, Strain 3D7

Catalog No. MRA-102

This reagent is the tangible property of the U.S. Government.

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 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands.

Lot[1]: 70017863 **Manufacturing Date: 07AUG2018**

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	11.5 ± 0.3 nM
Artemisinin	Report results	17.6 ± 0.4 nM
Quinine	Report results	40.5 ± 1.9 nM
Cycloguanil	Report results	8.2 ± 0.4 nM
Pyrimethamine	Report results	29.4 ± 1.4 nM
Sulfadoxine	Report results	476.6 ± 55.0 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943.1)	99.3% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943.1) (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	5.88%
Total parasitemia	≥ 2%	7.42%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	3.99%
Total parasitemia	≥ 1%	6.12%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells (Figure 2)
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[10], 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,

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

5% CO_2, 5% O_2) and monitored for parasitemia daily for 11 days. Every 2 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 11 days post infection by microscopic counts of Giemsa-stained blood smears.

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

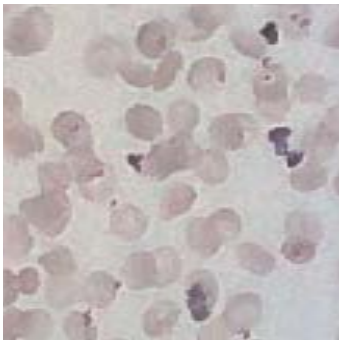
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.

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

Figure 1: MRA-102 MSP2 Sequence

TATAGCAACA CATTCATAAA CAATGCTTAT AAAGGGAAGT ATAAGGAGAA GTATGGCAGA AAGTAAGCCT TCTACTGGTG
CTGGTGGTAG TGCTGGTGGT AGTGCTGGTG GTAGTGCTGG TGGTAGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTTCT
GGTGATGGTA ATGGTGCAGA TGCTGAGGGA AGTTCAAGTA CTCCCGCTAC TACCACAACT ACCAAAACTA CCACAACTAC
CACAACTACT AATGATGCAG AAGCATCTAC CAGTACCTCT TCAGAAAATC CAAATCATAA AAATGCCGAA ACAAATCCAA
AAGGTAAAGG AGAAGTTCAA GAACCAAATC AAGCAAATAA AGAAACTCAA AATAACTCAA ATGTTCAACA AGACTCTCAA
ACTAAATCAA ATGTTCCACC CACTCAAGAT GCAGACACTA AAAGTCCTAC TGCACAACCT GAACAAGCTG AAAATTCTGC
TCCAACAGCC GAACAAACTG AATCCCCCGA ATTACAATCT GCACCAGAGA ATAAAGGTAC AGGACAACAT GGACATATGC
ATGGTTCTAG AAATAATCAT CCACAAAATA CTTCTGATAG TCAAAAAGAA TGTACCGATG GTAACAAAGA AAAACTGTGG
AGCAGCAACA TCCCTCTTAA ATAACTCTAG TAATATTGCT TCAATAAATA AATTTGTTGT TTTAATTTCA GCAACACTTT
G

Figure 2: Viability (post-freeze)



/Heather Couch/
Heather Couch 22 SEP 2018
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898