b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-155

Plasmodium falciparum, Strain HB3

Catalog No. MRA-155

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

Product Description: *Plasmodium falciparum (P. falciparum)*, strain HB3 was cloned from the Honduras I/CDC strain, originally isolated from a patient in Choluteca, Honduras, during an outbreak of urban malaria in January 1980.

Lot[1]: 63834914 **Manufacturing Date: 06NOV2015**

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 Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	 Report results Report results Report results Report results Report results Report results	 9.1 ± 0.3 nM 7.1 ± 0.5 nM 87.7 ± 11.3 nM 47.7 ± 5.7 nM 2744 ± 164.4 nM 473900 ± 52484.1 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs) MSP2 PCR amplicon analysis[5]	 Consistent with *P. falciparum* ~ 600-900 base pair amplicon	 Consistent with *P. falciparum*[4] (Figure 1) ~ 900 base pair amplicon (Figure 2)
Level of Parasitemia Pre-freeze[6] Post-freeze[7]	 Report results > 1%	 4.22% 5.08%
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 Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	 No growth No growth No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination DNA Detection by PCR	 None detected	 None detected

[1]MRA-155 was produced by cultivation of MR-MRA-155 lot 58243283 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 15 days. Every 2 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% 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 3 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.mr4.org/Publications/MethodsinMalariaResearch.aspx].

[4]100% sequence identity to GenBank: AANS01000284 (*P. falciparum*, strain HB3)

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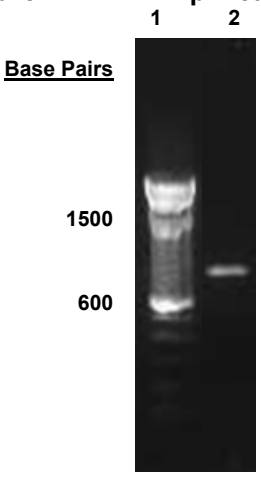
[5]Primer sequences and conditions for PCR are available upon request.
[6]Pre-freeze parasitemia was determined after 15 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 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-155 MSP2 Sequence

TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA AGAGTGTAGG TGCAAATGCT
CCAAAAGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAAAATACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG
AAAGTAATTC ACCTTCACCA CCCATCACTA CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA
AGTTCTGGCA ATGCACCAAA TAAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA
AGAAGGACCC AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC
AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC
AAAGAAAACT GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAAT
TTCAGCAACA CTTGTTTTAT CTTTTGC

Figure 2: PCR Amplification of MSP2



Lane 1: 100 base pair ladder
Lane 2: 100 ng of MRA-155

Date: 25 FEB 2016 **Signature:**

BEI Resources Authentication

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.

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