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]: 70014464 **Manufacturing Date: 17APR2018**

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	7.8 ± 0.4 nM
Artemisinin	Report results	14.8 ± 0.3 nM
Quinine	Report results	41.6 ± 1.0 nM
Cycloguanil	Report results	32.8 ± 0.8 nM
Pyrimethamine	Report results	2346 ± 54.0 nM
Sulfadoxine	Report results	579100 ± 107279 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain HB3 (GenBank: AANS01000284)	99.9% sequence identity to *P. falciparum*, strain HB3 (GenBank: AANS01000284) (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia		
Pre-freeze[6,7]		
Ring-stage parasitemia	Report results	5.57%
Total parasitemia	≥ 2%	6.89%
Post-freeze[2,8]		
Ring-stage parasitemia	Report results	0.90%
Total parasitemia	≥ 1%	1.25%
Viability (post-freeze)[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
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-155 was produced by cultivation of BEI Resources 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 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.

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[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 5 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 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 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-155 MSP2 Sequence

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ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAAACAAT GCTTATAATA TGAGTATAAG
GAGAAGTATG GCAAATGAAG GTTCTAATAC TAAGAGTGTA GGTGCAAATG CTCCAAAAGC TGATACTATT GCTAGTGGAA
GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAAATA CTACTCCTAC CGCTGCTGAT
ACCCCTACTG CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC
TACTACAAAA AGTAATTCAC CTTCACCACC CATCACTACT ACAGAAAGTT CAAGTTCTGG CAATGCACCA AATAAAACAG
ACGGTAAAGG AGAAGAGAGT GAAAAACAAA ATGAATTAAA TGAATCAACT GAAGAAGGAC CCAAAGCTCC ACAAGAACCT
CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA AAGGTACAGG ACAACATGGA CATATGCATG GTTCTAGAAA
TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT ACCGATGGTA ACAAAGAAAA CTGTGGAGCA GCAACATCCC
TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTGTTTTA ATTTCARCAA CACTTGTTTT ATCTTTT
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/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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