

***Plasmodium falciparum*, Strain HB3**

Catalog No. MRA-155

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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.

Lot¹: 61219195

Manufacturing Date: 27SEP2012

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>) 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	12.7 ± 0.3 nM 4.3 ± 0.6 nM 97.3 ± 4.5 nM 48.5 ± 4.5 nM 340.7 ± 23.6 nM 413800 ± 57350.6 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 base pairs) MSP2 PCR amplicon analysis ⁴	≥ 99% identical to GenBank: AANS01000284 (<i>P. falciparum</i> , strain HB3) ~ 600-900 base pair amplicon	99.1% sequence identity to GenBank: AANS01000284 (<i>P. falciparum</i> , strain HB3) (Figure 1) ~ 800 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Ring-stage parasitemia Total parasitemia Post-freeze ⁶ Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	5.55% 7.59% 1.26% 1.51%
Viability (post-freeze)⁷	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 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

¹MRA-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% N₂, 5% CO₂, 5% O₂) 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.

²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 2 days.

³A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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>].

⁴Primer sequences and conditions for PCR are available upon request.

⁵Pre-freeze parasitemia was determined after 17 days post infection by microscopic counts of Giemsa-stained blood smears.

⁶Post-freeze parasitemia was determined after 2 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 2 days post infection.

⁸Atlas, 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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TATGGCAAAA GA-TAAAAC- AAGTGTGCT GAAATTAATA CAACAAATTT ATTTATTGAA GCAATATTAC TAGAGTTATT
TAAGAGGGAT GTTGCTGCTC AWGAAGGTAA TTAAAACATT GTSTATTATA AATTTSTTTA TYTTTGTTAC CTTTAATATT
AAAAATGAAA GTAAATATAG CAACACATTC ATAAACAATG CTTATAATAT GAGTATAAGG AGAAGTATGG CAAATGAAGG
TTCTAATACT AAGAGTGTAG GTGCAAATGC TCCAAAAGCT GATACTATTG CTAGTGGAAG TCAAAGTAGT ACAAATAGTG
CAAGTACTAG TACTACTAAT AATGGAGAAT CACAAAATAC TACTCCTACC GCTGCTGATA CCCCTACTGC TACAGAAAGT
AATTCACCTT CACCACCCAT CACTACTACA GAAAGTAATT CACCTTCACC ACCCATCACT ACTACAAAAA GTAATTCACC
TTCACCACCC ATCACTACTA CAGAAAGTTC AAGTTCTGGC AATGCACCAA ATAAAACAGA CGGTAAAGGA GAAGAGAGTG
AAAAACAAAA TGAATTAAT GAATCAACTG AAGAAGGACC CAAAGCTCCA CAAGAACCTC AAACGGCAGA AAATGAAAAAT
CCTGCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATACTTC
TGATAGTCAA AAARAATGWA CCGATGGTAA CAAARAAAAA TGTGGAGCAG CAACATCCCT CTAAATAAC TCTA
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Date: 17 NOV 2017

Signature: 

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