

***Plasmodium falciparum*, Strain HB3B**

Catalog No. MRA-1227

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain HB3B is a subclone of the HB3 strain after mosquito and chimpanzee passage. *P. falciparum*, strain HB3 (available as BEI Resources MRA-155) 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: 63750689

Manufacturing Date: 20OCT2015

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	9.5 ± 0.4 nM 5.8 ± 0.1 nM 51.3 ± 2.4 nM 24.6 ± 1.7 nM 1327.0 ± 30.6 nM 550500 ± 25360 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs) MSP2 PCR amplicon analysis ⁴	≥ 99% sequence identity to <i>P. falciparum</i> , strain HB3 (GenBank: AANS01000284.1) ~ 600-900 base pair amplicon	100% sequence identity to <i>P. falciparum</i> , strain HB3 (GenBank: AANS01000284.1) (Figure 1) ~ 800 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Post-freeze ⁶	Report results > 1%	3.23% 2.67%
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-1227 was produced by cultivation of the deposited material 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 5 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% 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 5 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.mr4.org/Publications/MethodsInMalariaResearch.aspx>].

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

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

⁶Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 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-1227 MSP2 Sequence

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TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA AGAGTGTAGG TGCAAATGCT
CCAAAAGCTG ATACTATTGC TAGTGGAAAGT CAAAGTAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAAAATACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAAGTA ATTACCTTC ACCACCCATC ACTACTACAG
AAAGTAATTC ACCTTCACCA CCCATCACTA CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA
AGTTCTGGCA ATGCACCAAA TAAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAATG AATCAACTGA
AGAAGGACCC AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC
AACATGGACA TATGCATGGT TCTAGAAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC
AAAGAAAACGT GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAAT
TTCAGCAACA CTTGTTTTAT CTTTTG
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Date: 10 JUN 2016

Signature:



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