

Plasmodium falciparum, Strain 3BA6

Catalog No. MRA-165

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Product Description: *Plasmodium falciparum* (*P. falciparum*), strain 3BA6 is a genetic cross progeny of *P. falciparum* strains HB3 and Dd2.

Lot¹: 70017003

Manufacturing Date: 12JUL2018

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy^{2,3}	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)² Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ⁴ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	79.2 ± 7.3 nM 10.5 ± 1.0 nM 173 ± 20 nM 196.2 ± 22.6 nM 8704 ± 601.7 nM 369700 ± 34099 nM
Genotypic Analysis² Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	≥ 99% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: DS016061.1)	99.6% sequence identity to <i>P. falciparum</i> , strain Dd2 (GenBank: DS016061.1) (Figure 1)
Functional Activity by PCR Amplification² 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 Total parasitemia Post-freeze ^{2,8} Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	 2.03% 3.78% 0.58% 1.15%
Viability^{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 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 Contamination² DNA Detection by PCR	None detected	None detected

¹MRA-165 was produced by cultivation of BEI Resources MRA-165 lot 2518228 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 13 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.

²Testing completed on viald post-freeze material.

³Blood-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.

⁴A 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>].

⁵Primer sequences and conditions for PCR are available upon request.

⁶Testing completed on bulk material prior to vialing and freezing.

⁷Parasitemia was determined after 13 days post infection by microscopic counts of Giemsa-stained blood smears.

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

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

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

Figure 1: MRA-165 MSP2 Sequence

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CTTTAATATT  AAAAATGAAA  GTAAATATAG  CAACACATTC  ATAAACAATG  CTTATAATAT  GAGTATAAGG  AGAAGTATGG
CAAATGAAGG  TTCTAATACT  ACTAGTGTAG  GTGCAAATGC  TCCAAATGCT  GATACTATTG  CTAGTGGAAG  TCAAAGTAGT
ACAAATAGTG  CAAGTACTAG  TACTACTACT  AATAATGGAG  AATCACAAAC  TACTACTCCT  ACCGCTGCTG  ATACTATTGC
TAGTGGAAGT  CAAAGGAGTA  CAAATAGTGC  AAGTACTAGT  ACTACTAATA  ATGGAGAATC  ACAACTACT  ACTCCTACCG
CTGCTGATAC  TATTGCTAGT  GGAAGTCAAA  GGAGTACAAA  TAGTGCAAGT  ACTAGTACTA  CTAATAATGG  AGAATCACAA
ACTACTACTC  CTACCGCTGC  TGATACCCCT  ACTGCTACAG  AAAGTAATTC  ACCTTCACCA  CCCATCACTA  CTACAGAAAG
TTCAAGTTCT  GGCAATGCAC  CAAATAAAAC  AGACGGTAAA  GGAGAAGAGA  GTGAAAAACA  AAATGAATTA  AATGAATCAA
CTGAAGAAGG  ACCCAAAGCT  CCACAAGAAC  CTCAAACGGC  AGAAAATGAA  AATCCTGCTG  CACCAGAGAA  TAAAGGTACA
GGACAACATG  GACATATGCA  TGGTTCTAGA  AATAATCATC  CACAAAATAC  TTCTGATAGT  CAAAAAGAAT  GTACCGATGG
TAACAAAGAA  AACTGTGGAG  CAGCAACATC  CCTCTTAAAT  AACTCTAGTA  ATATTGCTTC  AATAAATAAA
    
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19 SEP 2018

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