

***Plasmodium falciparum*, Strain HB3-B2**

Catalog No. MRA-149

Product Description:

Plasmodium falciparum (*P. falciparum*), strain HB3-B2 was derived from clone HB3 after being transmitted through mosquitoes into a chimpanzee. Clone HB3 was derived from isolate H1 from Honduras by W. Trager by microscopic selection. MRA-149 lot 70055235 was produced by cultivation of BEI Resources seed lot 57692851 in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 µg/mL hypoxanthine and 5 µg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 28 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.

Lot: 70055235

Manufacturing Date: 07OCT2022

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR Green I [®] drug sensitivity assay ²		
Chloroquine	Report results	8.4 ± 0.4 nM
Artemisinin	Report results	17.9 ± 1.2 nM
Quinine	Report results	69.1 ± 4.8 nM
Cycloguanil	Report results	20.2 ± 1.4 nM
Pyrimethamine	Report results	5337 ± 492 nM
Sulfadoxine	Report results	529600 ± 24398 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (28 days post-infection) ³		
Ring-stage parasitemia	Report results	3.00%
Total parasitemia	≥ 2%	4.37%
Post-freeze (2 days post-infection) ¹		
Ring-stage parasitemia	Report results	2.62%
Total parasitemia	≥ 1%	3.18%
Viability (3 days post-infection)¹	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)¹		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud 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 Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vialled, post-freeze material

²A 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. *Methods in Malaria Research Sixth Edition* is available on the [BEI Resources website](#).]

³Testing completed on bulk material prior to vialing and freezing

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-149 MSP2 Sequence

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TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT
TCTAATACTA AGAGTGTAGG TGCAAATGCT CAAAAGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA CAAATAGTGC AAGTACTAGT
ACTACTAATA ATGGAGAATC ACAAATACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAAGTA ATTCACCTTC ACCACCCATC
ACTACTACAG AAAGTAATTC ACCTTCACCA CCCATCACTA CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA
AGTTCTGGCA ATGCACCAA TAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA AGAAGGACCC
AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT
TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAA AAGAATGTAC CGATGGTAAAC AAAGAAAAC GTGGAGCAGC AACATCCCTC
TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAAT TTCAGCAACA CTTGTTTTAT CTTTTGC
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