SUPPORTING INFECTIOUS DISEASE RESEARCH

Plasmodium falciparum, Strain SB1-A6

Catalog No. MRA-1002

Product Description:

Plasmodium falciparum (*P. falciparum*), strain SB1-A6 is a drug-selected [3-(6,6,6-trifluorohexyloxy)-6-aminoacridone (6-NH2Ac)] clone of the parent strain D6. Strain D6 was collected in Sierra Leone. MRA-1002 lot 70050432 was produced by cultivation of the BEI Resources seed lot 59073395 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, 2 g/L D-glucose, 27 μ g/mL hypoxanthine and 5 μ g/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 for 12 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70050432

Manufacturing Date: 01MAR2022

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| TEST | SPECIFICATIONS | RESULTS |
|---|------------------------------------|------------------------------------|
| Identification by Giemsa Stain Microscopy ¹ | 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 | 6.6 ± 0.5 nM |
| Artemisinin | Report results | 12.2 ± 0.3 nM |
| Quinine | Report results | 29.5 ± 1.4 nM |
| Cycloguanil | Report results | 8.9 ± 1.2 nM |
| Pyrimethamine | Report results | 28.2 ± 2.6 nM |
| Sulfadoxine | Report results | 568700 ± 39316 nM |
| Atovaquone | | |
| 24.4 nM to 25,000 nM | Report results | 7561 ± 348 nM |
| 0.24 nM to 250 nM | Report results | 78 ± 1.6 nM |
| Genotypic Analysis ¹ | · · | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) | Consistent with P. falciparum | Consistent with P. falciparum |
| gene (~ 710 base pairs) | | (Figure 1) |
| Level of Parasitemia by Giemsa Stain Microscopy | | |
| Pre-freeze (12 days post-infection) ³ | | |
| Ring-stage parasitemia | Report results | 3.09% |
| Total parasitemia | ≥ 2% | 5.06% |
| Post-freeze (3 days post-infection) ¹ | | |
| Ring-stage parasitemia | Report results | 0.58% |
| Total parasitemia | ≥ 1% | 2.14% |
| 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 |

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Certificate of Analysis for MRA-1002

SUPPORTING INFECTIOUS DISEASE RESEARCH

| TEST | SPECIFICATIONS | RESULTS |
|---|----------------|---------------|
| Mycoplasma Contamination ¹ DNA detection by PCR | None detected | None detected |

¹Testing completed on vialed, 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 <u>Methods in Malaria Research Sixth Edition</u>. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. <u>Methods in Malaria Research Sixth Edition</u> is available on the <u>BEI Resources website</u>.]
³Testing completed on bulk material prior to vialing and freezing

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

Figure 1: MRA-1002 MSP2 Sequence

CTTTAATATT AAAAATGAAA GTAAATATAG CAACACATTC ATAAACAATG CTTATAATAT GAGTATAAGG AGAAGTATGG CAAATGAAGG TTCTAGTACT AATAGTGTAG GTGCAAATGC TCCAAAAGCT GATACTATTG CTAGTGGAAG TCAAAGTAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATAGAGAAT CACAAACTAC TACTCCTACC ACTGCTGATA CCCCTACTGC TACAGAAAGT AATTCACCTT CACCACCAT CGCTACTACA GAAAGTAATT CACCTTCACC ACCCATCACT ACTACAGAAA GTAATTCACC TTCACCACCC ATCACTACTA CAGAAAGTTC AAGTTCTGGC AATGCACCAA ATAAAACAGA CGGTAAAGGA GAAGAGAGTG AAAAACAAAA TGAATTAAAT GAATCAACTG AAGAAAGTC CAAAGCTCCA CAAGAACCTC AAACGGCAGA AAATGAAAAT CCTGCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA CAAAGAAAAC TGTGGAGCAG CAACATCCCT CTTAAATAAC TCTAGTAATA TTGCTTCAAT AAATAAATTT GTTGTTTTAA TTTCAGCAAC ACTTGTTTTA TCTTTT

/Sonia Bjorum Brower/

Sonia Bjorum Brower

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