

Plasmodium falciparum, Strain D6

Catalog No. MRA-285

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain D6 was collected in Sierra Leone and is generally considered drug sensitive with minor mefloquine resistance. MRA-285 was produced by cultivation of BEI Resources MR-MRA-285 lot 59155175 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 12 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: 70030910

Manufacturing Date: 19NOV2019

| 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 | 7.6 ± 0.2 nM |
| Artemisinin | Report results | 26.9 ± 0.6 nM |
| Quinine | Report results | 20.7 ± 0.5 nM |
| Cycloguanil | Report results | 7.5 ± 0.5 nM |
| Pyrimethamine | Report results | 26.2 ± 1.8 nM |
| Sulfadoxine | Report results | 565700 ± 39108 nM |
| Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (760 base pairs) | ≥ 99% sequence identity to <i>P. falciparum</i> , strain D6 (GenBank: ABGY01002422.1) | 99.9% sequence identity to <i>P. falciparum</i> , strain D6 (GenBank: ABGY01002422.1) (Figure 1) |
| MSP2 PCR amplicon analysis ¹ | ~ 600-900 base pair amplicon | ~ 900 base pair amplicon |
| Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (12 days post-infection) ³ | | |
| Ring-stage parasitemia | Report results | 2.58% |
| Total parasitemia | ≥ 2% | 4.38% |
| Post-freeze (2 days post-infection) ¹ | | |
| Ring-stage parasitemia | Report results | 2.00% |
| Total parasitemia | ≥ 1% | 3.14% |
| Viability (post-freeze; 2 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 Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud 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 Contamination¹ DNA detection by PCR | None detected | None detected |

¹Testing completed on vial, 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. Available at: <https://www.beiresources.org/Publications/MethodsInMalariaResearch.aspx>]

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-285 MSP2 Sequence

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ATTAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT
CATAAACAAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAAATGAAG GTTCTAGTAC TAATAGTGTA GGTGCAAATG
CTCCAAAAGC TGATACTATT GCTAGTGGAA GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATAGAGAA
TCACAAACTA CTA CTACTCCTAC CACTGCTGAT ACCCCTACTG CTACAGAAAAG TAATTCACCT TCACCACCCA TCGCTACTAC
AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTAATTCAC CTTCAACCACC CATCACTACT ACAGAAAGTT
CAAGTTCTGG CAATGCACCA AATAAAACAG ACGGTAAAGG AGAAGAGAGT GAAAAACAAA ATGAATTAAA TGAATCAACT
GAAGAAGGAC CCAAAGCTCC ACAAGAACCT CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA AAGGTACAGG
ACAACATGGA CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT ACCGATGGTA
ACAAAGAAAA CTGTGGAGCA GCAACATCCC TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTGTTTTA
ATTCAGCAA CACTTGTTTT ATCTTTTGCC ATATTCATAT
    
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15 JUL 2020

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