

***Plasmodium falciparum*, Strain 3D7**

Catalog No. MRA-102

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain 3D7 was cloned from the NF54 strain by limiting dilution; it is reported as a pyrimethamine-sensitive strain. The parent NF54 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands. MRA-102 was produced by cultivation of the BEI Resources seed 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 14 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: 70032033

Manufacturing Date: 23JAN2020

| 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 | 6.1 ± 0.3 nM |
| Artemisinin | Report results | 6 ± 0.4 nM |
| Quinine | Report results | 43.2 ± 2 nM |
| Cycloguanil | Report results | 11.9 ± 0.5 nM |
| Pyrimethamine | Report results | 47.6 ± 3.3 nM |
| Sulfadoxine | Report results | 228100 ± 26319 nM |
| Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 810 base pairs) | ≥ 99% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1) | 100% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1) (Figure 1) |
| Functional Activity by PCR Amplification¹ MSP2 PCR amplicon analysis | ~ 600-900 base pair amplicon | ~ 900 base pair amplicon |
| Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (14 days post-infection) ³ | | |
| Ring-stage parasitemia | Report results | 3.31% |
| Total parasitemia | ≥ 2% | 5.72% |
| Post-freeze (4 days post-infection) ¹ | | |
| Ring-stage parasitemia | Report results | 1.50% |
| Total parasitemia | ≥ 1% | 4.80% |
| Viability (post-freeze; 4 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 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. Available at: to <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-102 MSP2 Sequence

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TTAAAACATT GTCTATTATA AATTTCTTTA TTTTTGTTAC CTTTAATATT AAAAATGAAA GTAAATATAG CAACACATTC
ATAACAATG CTTATAATAT GAGTATAAGG AGAAGTATGG CAGAAAGTAA GCCTTCTACT GGTGCTGGTG GTAGTGCTGG
TGGTAGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTGGT AGTGCTGGTG GTAGTGCTGG TTCTGGTGAT GGTAATGGTG
CAGATGCTGA GGAAGTTCA AGTACTCCCG CTACTACCAC AACTACCAA ACTACCACAA CTACCACAA TACTAATGAT
GCAGAAGCAT CTACCAGTAC CTCTTCAGAA AATCCAAATC ATAAAAATGC CGAAACAAAT CAAAAGGTA AAGGAGAAGT
TCAAGAACCA AATCAAGCAA ATAAAGAAAC TCAAAATAAC TCAAATGTTT AACAAGACTC TCAAATAAA TCAAATGTTT
CACCCACTCA AGATGCAGAC ACTAAAAGTC CTACTGCACA ACCTGAACAA GCTGAAAATT CTGCTCCAAC AGCCGAACAA
ACTGAATCCC CCGAATTACA ATCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT ATGCATGGTT CTAGAAATAA
TCATCCACAA AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGAAAAC TGAGCAGCA ACATCCCTCT
TAAATAACTC TAGTAATATT GCTTCAATAA ATAAATTTGT TGTTTTAATT TCAGCAACAC TTGTTTTATC TTTTGCCATA
TTCATATAAA
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07 JUL 2020

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