

Plasmodium falciparum, Strain 3D7HT-GFP

Catalog No. MRA-1029

Product Description:

Plasmodium falciparum (*P. falciparum*), strain 3D7HT-GFP is was created by stable transfection of the parent 3D7 strain with a plasmid containing the green fluorescent protein (GFP) under control of the EF1 α promoter and integration into the Pf47 locus of chromosome 13. *P. falciparum*, strain 3D7 (available as BEI Resources MRA-102) was originally isolated in the Netherlands. MRA-1029 lot 70027878 was produced by cultivation of BEI Resources seed lot 59155174 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 μ g/mL hypoxanthine and 2.5 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 every 1 to 3 days for 35 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: 70027878

Manufacturing Date: 17SEP2019

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	11.7 \pm 0.3 nM
Artemisinin	Report results	6.9 \pm 0.2 nM
Quinine	Report results	157.8 \pm 10.9 nM
Cycloguanil	Report results	67.5 \pm 12.5 nM
Pyrimethamine	Report results	36.5 \pm 5.9 nM
Sulfadoxine	Report results	488700 \pm 45075 nM
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 810 base pairs)	\geq 99% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1)	99.9% 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	3.77%
Total parasitemia	\geq 2%	6.28%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	4.9%
Total parasitemia	\geq 1%	5.25%
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	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 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: 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-1029 MSP2 Sequence

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ATGAAGGTAA TTAAACATT TGTCTATTAT AAATTTCTTT ATTTTGTGTT CCTTTAATAT TAAAAATGAA AGTAAATATA
GCAACACATT CATAACAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAGAAAGTA AGCCTTCTAC TGGTGCTGGT
GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTTCTGGTGA
TGGTAATGGT GCAGATGCTG AGGGAAGTTC AAGTACTCCC GCTACTACCA CAACTACCAA AACTACCACA ACTACCACAA
CTACTAATGA TGCAGAAGCA TCTACCAGTA CCTCTTCAGA AAATCCAAAT CATAAAAATG CCGAAACAAA TCCAAAAGGT
AAAGGAGAAG TTCAAGAACC AAATCAAGCA AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT CTCAAATAA
ATCAAATGTT CCACCCACTC AAGATGCAGA CACTAAAAGT CCTACTGCAC AACCTGAACA AGCTGAAAAT TCTGCTCCAA
CAGCCGAACA AACTGAATCC CCCGAATTAC AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT
TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAACGT GTGGAGCAGC
AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAAT TTCAGCAACA CTTGTTTTAT
CTTTTGCC
    
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27 AUG 2020

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