

***Plasmodium falciparum*, Strain 3D7A**

Catalog No. MRA-151

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain 3D7A is a subclone of the 3D7 strain. *P. falciparum*, strain 3D7 (available as BEI Resources MRA-102) was cloned from the NF54 strain by limiting dilution. The parent NF54 isolate was derived from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands. MRA-151 was deposited as chloroquine- and pyrimethamine-sensitive. *P. falciparum* 3D7A is used as a standard for the *P. falciparum* genome sequencing project and as a parent clone of the 3D7/HB3 cross.

Lot¹: 2164

Manufacturing Date: 15NOV2016

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>) Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ³ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	7.3 ± 0.7 nM 4.2 ± 0.2 nM 121.2 ± 16.8 nM 9.1 ± 0.6 nM 33.4 ± 2.3 nM 388000 ± 35787 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 base pairs) MSP2 PCR amplicon analysis ⁴	≥ 99% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1) ~ 600-900 base pair amplicon	100% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: LN999943.1) (Figure 1) ~ 900 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Ring-stage parasitemia Total parasitemia Post-freeze ⁶ Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	3.52% 4.34% 2.78% 5.00%
Viability (post-freeze)⁷	Growth in infected red blood cells	Growth in infected red blood cells (Figure 2)
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose 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 Contamination DNA Detection by PCR	None detected	None detected

¹MRA-151 was produced by cultivation of MR-MRA-151 lot 59531004 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 7 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.

²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 4 days.

³A SYBR Green I[®] antimalarial 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>].

⁴Primer sequences and conditions for PCR are available upon request.

⁵Pre-freeze parasitemia was determined after 7 days post infection by microscopic counts of Giemsa-stained blood smears.

⁶Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.

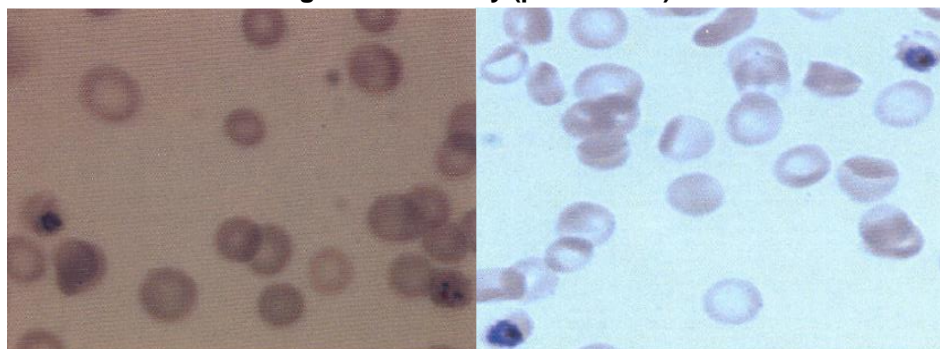
⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-151 MSP2 Sequence

```

ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAAACAAT GCTTATAATA TGAGTATAAG
GAGAAGTATG GCAGAAAGTA AGCCTTCTAC TGGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA
GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTTCTGGTGA TGGTAATGGT GCAGATGCTG AGGGAAGTTC AAGTACTCCC
GCTACTACCA CAACTACCAA AACTACCACA ACTACCACAA CTAATAATGA TGCAGAAGCA TCTACCAGTA CCTCTTCAGA
AAATCCAAAT CATAAAAATG CCGAAAACAAA TCCAAAAGGT AAAGGAGAAG TTCAAGAACC AAATCAAGCA AATAAAGAAA
CTCAAAATAA CTCAAAATGTT CAACAAGACT CTCAAATAA ATCAAATGTT CCACCCACTC AAGATGCAGA CACTAAAAGT
CCTACTGCAC AACCTGAACA AGCTGAAAAT TCTGCTCCAA CAGCCGAACA AACTGAATCC CCCGAATTAC AATCTGCACC
AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA
AAGAATGTAC CGATGGTAAC AAAGAAAAC TGTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA
AATAAATTTG TTGTTT
    
```

Figure 2: Viability (post-freeze)



Date: 21 JUL 2017

Signature:

BEI Resources Authentication

ATCC[®], on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC[®]'s knowledge.

ATCC[®] is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

