

***Plasmodium falciparum*, Strain 3D7 (+cas9)**

**Catalog No. MRA-1327**

**Product Description:**

*Plasmodium falciparum* (*P. falciparum*), strain 3D7 (+cas9) was deposited to BEI Resources as a transgenic strain that expresses the Cas9 protein. Strain 3D7 (+cas9) was produced by integrating Cas9 expression cassette at the *kahrp* locus of *P. falciparum*, strain 3D7 (BEI Resources MRA-102). MRA-1327 was produced by cultivation of deposited material in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 µg/mL hypoxanthine and 5 µg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with a blood-gas atmosphere (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia for 20 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: 70058641**

**Manufacturing Date: 25MAY2023**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>1</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Genotypic Analysis<sup>2</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 base pairs)	Consistent with <i>P.falciparum</i>	Consistent with <i>P.falciparum</i> (Figure 1)
<b>Level of Parasitemia by Giemsa Stain Microscopy</b> Pre-freeze (20 days post-infection) <sup>2</sup> Ring-stage parasitemia Total parasitemia Post-freeze (2 days post-infection) <sup>1</sup> Ring-stage parasitemia Total parasitemia	Report results ≥ 2%  Report results ≥ 1%	3.3% 4.6%  6.8% 8.8%
<b>Viability (1 day post-infection)<sup>1</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)<sup>1</sup></b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup> 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
<b>Mycoplasma Contamination<sup>1</sup></b> DNA detection by PCR	None detected	None detected

<sup>1</sup>Testing completed on vial, post-freeze material

<sup>2</sup>Testing completed on bulk material prior to vialing and freezing

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

**Figure 1: MRA-1327 MSP2 Gene Sequence**

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AAAACATTGTCTATTATAAATTTCTTTATTTTTGTTACCTTTAATATTTAAAAATGAAAGTAAATATAGCAAC
ACATTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCAGAAAGTAAGCCTTCTACTGGTGCT
GGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCT
GGTTCTGGTGATGGTAATGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCGCTACTACCACAACACTACCAA
ACTACCACAACACTACCACAACACTACTAATGATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCAT
AAAAATGCCGAAACAAATCCAAAAGGTAAAGGAGAAGTTCAAGAACCAAATCAAGCAAATAAAGAAACTCAA
AATAACTCAAATGTTCAACAAGACTCTCAAATAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAA
AGTCCTACTGCACAACCTGAACAAGCTGAAAATTTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTA
CAATCTGCACCAGAGAATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAA
AATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAGAAAACACTGTGGAGCAGCAACATCCCTCTTA
AATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTTCAGCAACACTTGTTTTATCTTTT
    
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Technical Manager or designee, ATCC Federal Solutions

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