

***Plasmodium falciparum*, Strain 3D7-B E5-10.3-Tom**

**Catalog No. MRA-1306**

**Product Description:**

*Plasmodium falciparum* (*P. falciparum*), strain 3D7-B E5-10.3-Tom was deposited to BEI Resources as a fluorescent, transgenic strain derived from the high gametocyte-producing strain E5, which is a subclone of the 3D7-B line. Strain 3D7-B E5-10.3-Tom was generated through the integration of a cassette containing the fluorescent reporter gene *tdTomato*, under the control of the *etramp 10.3* promoter, inserted at the liver-specific protein-1 (*lisp1*) gene locus using CRISPR-Cas9 technology. MRA-1306 was produced by cultivation of BEI Resources seed lot 70059847 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, 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 blood-gas atmosphere (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia every 1 to 3 days for 21 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: 70067305**

**Manufacturing Date: 12MAR2024**

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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>1</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 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 (21 days post-infection) <sup>2</sup> Ring-stage parasitemia Total parasitemia Post-freeze (3 days post-infection) <sup>1</sup> Ring-stage parasitemia Total parasitemia	Report results ≥ 2%	2.4% 4.2%
<b>Phenotypic Analysis</b> <i>tdTomato</i> expression <sup>3</sup>	Positive	Positive
<b>Viability (3 days post-infection)<sup>1</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (14-day incubation)<sup>1</sup></b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>4</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 vialled, post-freeze material

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

<sup>3</sup>*tdTomato* expression was examined by fluorescence microscopy.

<sup>4</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

