

***Plasmodium falciparum*, Strain NF54-10.3-Tom**

Catalog No. MRA-1305

Product Description:

Plasmodium falciparum (*P. falciparum*), strain NF54-10.3-Tom was deposited to BEI Resources as a fluorescent, transgenic strain, and derived from the high gametocyte-producing strain NF54. Strain NF54-10.3-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-1305 was produced by cultivation of BEI Resources seed lot 70059844 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia every 1 to 3 days for 25 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: 70065845

Manufacturing Date: 29JAN2024

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 730 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (25 days post-infection) ² Ring-stage parasitemia Total parasitemia Post-freeze (2 days post-infection) ¹ Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	1.9% 2.9% 4.5% 5.3%
Phenotypic Analysis <i>tdTomato</i> expression ³	Positive	Positive
Viability (2 days post-infection)¹	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (14-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

²Testing completed on bulk material prior to vialing and freezing

³*tdTomato* expression was examined by fluorescence microscopy.

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

Figure 1: MRA-1305 MSP2 Sequence

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TTTGTTACCTTTAATATTA AAAATGAAAGTAAATATAGCAACACATTCATAAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCAGAAAGTA
AGCCTTCTACTGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCCTACTACCACAAC TACCAAAACTACCACAAC TACCACAAC TACTAATGATGCA
TGATGGTAATGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCCTACTACCACAAC TACCAAAACTACCACAAC TACCACAAC TACTAATGATGCA
GAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAACAAATCCAAAAGGTAAAGGAGAAGTTC AAGAACCAAATCAAGCAA
ATAAAGAAACTCAAAATAACTCAAATGTTCAACAAGACTCTCAAAC TAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCTACTGC
ACAACCTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAATAAAGGTACAGGACAACAT
GGACATATGCATGGTTC TAGAAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAAGAAAAC TGTGGAGCAGCAA
CATCCCTCTTAATAACTCTAGTAATATGCTTCAATAAATAAATTTGTT
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02 JUL 2024

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