

***Plasmodium falciparum*, Strain 3D7, Clone 10.2**

Catalog No. MRA-1271

Product Description:

MRA-1271 is a gametocyte-deficient clonal parasite line derived by targeted disruption of Pfs230 in *Plasmodium falciparum* strain 3D7. MRA-1271 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia 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.

Lot: 63449603

Manufacturing Date: 04MAY2015

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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 (~ 780 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
Phenotypic Analysis Gametocytemia (<i>in vitro</i>) ² Pfs230 expression (immunofluorescence assay) ³	Report results Report results	< 0.01% Negative
Level of Parasitemia by Giemsa Stain Microscopy Pre-freeze (7 days post-infection) ⁴ Ring-stage parasitemia Total parasitemia Post-freeze (3 days post-infection) ¹ Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	1.78% 2.96% 1.41% 4.21%
Viability (3 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 ⁵ 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.

²Gametocytemia was <0.01% after 18 days of *in vitro* growth, confirming that MRA-1271 should not be classified as a gametocyte-producing strain.

³Using monoclonal anti-*Plasmodium falciparum*, 3D7 230 kDa Gamete Surface Protein (BEI Resources MRA-878A) as primary antibody (1:250) and Goat anti-Mouse IgG (H+L) (Invitrogen™ F-2761) as secondary antibody (1:500).

⁴Testing completed on bulk material prior to vialing and freezing.

⁵Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1271 MSP2 Sequence

TATTATAAATTTCTTTATTTTTGTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACATTCATAAAACAATGCTTATAATATGAGTATAAGG
AGAAGTATGGCAGAAAGTAAGCCTTCTACTGGTGTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTG
GTGGTAGTGCTGGTTCGGTGATGGTAATGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCGCTACTACCACAAC TACCAAAACTACCACAAC TAC
CACAAC TACTAATGATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAAACAAATCCAAAAGGTAAGGAGAAGTT
CAAGAACCAAAATCAAGCAAATAAAGAAACTCAAATAACTCAAATGTTCAACAAGACTCTCAAAC TAAATCAAATGTTCCACCCACTCAAGATGCAG
ACACTAAAAGTCCTACTGCACAACCTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAA
TAAAGGTACAGGACAACATGGACATATGCATGGTTCAGAAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAA
GAAAAC TGTGGAGCAGCAACATCCCTCTAAATAACTCTAGTAATATTGCTTCAATAAAATAAATTTGTTGTTTTAATTTTCAGCAACACTTGTTTTAT
CTTTT

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