Certificate of Analysis for MRA-1272

Plasmodium falciparum, Strain 3D7, Clone n230

Catalog No. MRA-1272

Product Description:

MRA-1272 is a gametocyte-overproducing clonal parasite line derived by targeted disruption of Pfs230 in *Plasmodium falciparum* strain 3D7. MRA-1272 lot 63449606 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_2, 5% CO_2, 5% O_2) and monitored for parasitemia for 6 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: 63449606 Manufacturing Date: 28APR2015

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Phenotypic Analysis		
Gametocytemia (*in vitro*)[2]	Report results	5-6%
Pfs230 expression (immunofluorescence assay)[3]	Report results	Negative
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (6 days post-infection)[4]		
Ring-stage parasitemia	Report results	1.98%
Total parasitemia	≥ 2%	3.08%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	5.52%
Total parasitemia	≥ 1%	5.58%
Viability (2 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]	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination[1]		
DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material.
[2]Gametocytemia was observed after 18 days of *in vitro* growth. All five stages of gametocytes were present in the culture.
[3]Using 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).
[4]Testing completed on bulk material prior to vialing and freezing.
[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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Figure 1: MRA-1272 MSP2 Sequence

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TATTATAAATTTCTTTATTTTTGTTACCTTTAATATTAAAAATGAAAGTAAATATAGCAACACATTCATAAACAATGCTTATAATATGAGTATAAGG
AGAAGTATGGCAGAAAGTAAGCCTTCTACTGGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGGTGGTAGTGCTGG
GTGGTAGTGCTGGTTCTGGTGATGGTAATGGTGCAGATGCTGAGGGAAGTTCAAGTACTCCCGCTACTACCACAACTACCAAAACTACCACAACTAC
CACAACTACTAATGATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAACAAATCCAAAAGGTAAAGGAGAAGTT
CAAGAACCAAATCAAGCAAATAAAGAAACTCAAAATAACTCAAATGTTCAACAAGACTCTCAAACTAAATCAAATGTTCCACCCACTCAAGATGCAG
ACACTAAAAGTCCTACTGCACAACCTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGAA
TAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTCAAAAAGAATGTACCGATGGTAACAAA
GAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATATTGCTTCAATAAATAAATTTGTTGTTTTAATTTCAGCAACACTTGTTTTAT
CTTTT
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/Sonia Bjorum Brower/
Sonia Bjorum Brower 25 SEP 2023
Technical Manager or designee, ATCC Federal Solutions

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BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898