Certificate of Analysis for MRA-1288

Plasmodium falciparum, Strain Palo Alto, Clone 89F5, Variant O (VarO)

Catalog No. MRA-1288

Product Description:

MRA-1288 is a clonal line of *Plasmodium falciparum* (*P. falciparum*) Palo Alto 89F5 and is a varO antigenic variant. It expresses the varO gene encoding *P. falciparum* erythrocyte membrane protein 1, which forms rosettes and autocoagulates in the peripheral blood of splenectomized animals and is associated with severe malaria in children in Africa. MRA-1288 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 28 days. Every 1 to 4 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70028494 **Manufacturing Date: 08OCT2019**

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR Green I® drug sensitivity assay[2]		
Chloroquine	Report results	7.8 ± 0.7 nM
Artemisinin	Report results	6.9 ± 0.3 nM
Quinine	Report results	63.6 ± 5.9 nM
Cycloguanil	Report results	40.9 ± 4.7 nM
Pyrimethamine	Report results	1267 ± 116.9 nM
Sulfadoxine	Report results	296800 ± 34245 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (28 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.77%
Total parasitemia	≥ 2%	4.16%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.23%
Total parasitemia	≥ 1%	2.05%
Viability (1 day 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[4]	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

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TEST	SPECIFICATIONS	RESULTS
Mycoplasma Contamination[1] DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material
[2]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (> 70% ring stage) parasite culture in the presence of each antimalarial drug [Hartwig, C. L., et al. "XI: I. SYBR Green I®-Based Parasite Growth Inhibition Assay for Measurement of Antimalarial Drug Susceptibility in *Plasmodium falciparum*." In: Methods in Malaria Research Sixth Edition. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]
[3]Testing completed on bulk material prior to vialing and freezing
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1288 MSP2 Sequence

TATGAAGGGTAATTAAAACATTGTSTATTATAAATTTCTTTATTTTTGTTACCTTTAATATTAAAAATGAAA
GTAAATATAGCAACACATTCATAAACAATGCTTATAATATGAGTATAAGGAGAAGTATGGCAGAAAGTAAGC
CTCCTACTGGTACTGGTGGTAGTGGTAGTGCTGGTTCTGGTGCTGGTGCTAGTGCTGGTAATGGTGCTAATC
CTGGTGCAGATGCTGAGAGAAGTCCAAGTACTCCCGCTACTCCCGCTACTCCCGCTACTACCACAACTACCA
CAACTACTAATGATGCAGAAGCATCTACCAGTACCTCTTCAGAAAATCCAAATCATAAAAATGCCGAAACAA
ATCCAAAAGGTAAAGGAGAAGTTCAAAAACCAAATCAAGCAAATAAAGAAACTCAAAATAACTCAAATGTTC
AACAAGACTCTCAAACTAAATCAAATGTTCCACCCACTCAAGATGCAGACACTAAAAGTCCTACTGCACAAC
CTGAACAAGCTGAAAATTCTGCTCCAACAGCCGAACAAACTGAATCCCCCGAATTACAATCTGCACCAGAGA
ATAAAGGTACAGGACAACATGGACATATGCATGGTTCTAGAAATAATCATCCACAAAATACTTCTGATAGTC
AAAAAGAATGTACCGATGGTAACAAAGAAAACTGTGGAGCAGCAACATCCCTCTTAAATAACTCTAGTAATA
TTGCTTCAATAAATAAATTTGTTGTTTTAATTTCAGCAACACTTGTTTTATCTTTTGCCATATTCATATAAA

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/Sonia Bjorum Brower/
Sonia Bjorum Brower 21 APR 2023
Technical Manager or designee, ATCC Federal Solutions

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