

Certificate of Analysis for MRA-201

Plasmodium falciparum, Strain D10

Catalog No. MRA-201

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain D10 was isolated in Papua New Guinea and is generally considered drug sensitive.

Lot¹: 63834913

Manufacturing Date: 30OCT2015

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>) Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ³ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	7.1 ± 0.7 nM 5.8 ± 0.7 nM 548.4 ± 88.8 nM 8.0 ± 0.9 nM 37.2 ± 6.0 nM 474000 ± 54692 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 base pairs) MSP2 PCR amplicon analysis ⁴	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon	Consistent with <i>P. falciparum</i> (Figure 1) ~ 900 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Post-freeze ⁶	Report results > 1%	4.16% 3.44%
Viability (post-freeze)⁷	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation) Harpo's HTYE broth ⁸ , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose 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 Contamination DNA Detection by PCR	None detected	None detected

¹MRA-201 was produced by cultivation of MR-MRA-201 lot 60369606 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, 4 g/L D-glucose, 0.005 µg/mL hypoxanthine and 2.5 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 daily for 14 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% hematocrit.

²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.

³A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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. Available at: <https://www.mr4.org/Publications/MethodsInMalariaResearch.aspx>].

⁴Primer sequences and conditions for PCR are available upon request.

⁵Pre-freeze parasitemia was determined after 14 days post infection by microscopic counts of Giemsa-stained blood smears.

⁶Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.

⁸Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-201 MSP2 Sequence

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TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAAATGCTT
ATAATATGAG TATAAGGAGA AGTATGGCAA ATGAAGGTTC TAATACTAAT AGTGTAGGTG CAAATGCTCC AAATGCTGAT
ACTATTGCTA GTGGAAGTCA AAGGAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACACTACT
TCCTACCGCT GCTGATACTA TTGCTAGTGG AAGTCAAAGG AGTACAAATA GTGCAAGTAC TAGTACTACT AATAATGGAG
AATCACAAAC TACTACTCCT ACCGCTGCTG ATACCCCTAC TGCTACAGAA AGTAATTCAC CTTACCACC CATCACTACT
ACAGAAAGTT CAAGTTCTGG CAATGCACCA AATAAAACAG ACGGTAAAGG AGAAGAGAGT GAAAAACAAA ATGAATTAAA
TGAATCAACT GAAGAAGGAC CCAAAGCTCC ACAAGAACCT CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA
AAGGTACAGG ACAACATGGA CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT
ACCGATGGTA ACAAAGAAAA CTGTGGAGCA GCAACATCCC TCTTAAGTAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT
TGTTGTTTTA ATTTTC
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Date: 24 MAR 2016

Signature:



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