Certificate of Analysis for MRA-914

Plasmodium falciparum, Strain FC27/PNG

Catalog No. MRA-914

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain FC27/PNG (Papua New Guinea) was originally collected from a 4-year-old child with clinical malaria. Venous blood was collected aseptically into bottles containing heparin in Madang Hospital, Madang Province, Papua New Guinea. The FC27 strain is reported to be chloroquine-sensitive.

Lot[1]: 58572346 **Manufacturing Date: 21APR2009**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis[3] Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs) MSP2 PCR amplicon analysis[4]	Consistent with *P. falciparum* ~ 600-900 base pair amplicon	Consistent with *P. falciparum* (Figure 1) ~ 900 base pair amplicon
Level of Parasitemia Pre-freeze[5] Post-freeze[6]	 Report results > 1%	 3% rings 3% rings
Viability (post-freeze)[7]	Growth in infected red blood cells	Growth in infected red blood cells
Mycoplasma Contamination DNA Detection by PCR	 None detected	 None detected

[1]MRA-914 was produced by cultivation of MR-MRA-914 lot 58399822 in fresh human erythrocytes 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily. Uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture, as necessary, to maintain 2% hematocrit. In order to remove contaminating *Mycoplasma arginini*, the initial culture was treated with mycoplasma removal agent for 10 days.
[2]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 3 days.
[3]The genotypic analysis for this lot was performed on 14MAR2016.
[4]Primer sequences and conditions are available upon request.
[5]Pre-freeze parasitemia was determined after 14 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 days post infection.

Figure 1: MRA-914 MSP2 Sequence

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TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA ATAGTGTAGG TGCAAATGCT
CCAAATGCTG ATACTATTGC TAGTGGAAGT CAAAGGAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAAACTACT ACTCCTACCG CTGCTGATAC TATTGCTAGT GGAAGTCAAA GGAGTACAAA TAGTGCAAGT ACTAGTACTA
CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC TGATACCCCT ACTGCTACAG AAAGTAATTC ACCTTCACCA
CCCATCACTA CTACAGAAAG TTCAAGTTCT GGCAATGCAC CAAATAAAAC AGACGGTAAA GGAGAAGAGA GTGAAAAACA
AAATGAATTA AATGAATCAA CTGAAGAAGG ACCCAAAGCT CCACAAGAAC CTCAAACGGC AGAAAATGAA AATCCTGCTG
CACCAGAGAA TAAAGGTACA GGACAACATG GACATATGCA TGGTTCTAGA AATAATCATC CACAAAATAC TTCTGATAGT
CAAAAAGAAT GTACCGATGG TAACAAAGAA AACTGTGGAG CAGCAACATC CCTCTTAAGT AACTCTAGTA ATATTGCTTC
AATAAATAAA TTTGTTGTTT TAATTTCAGC AACACTTGTT TTATCTTTT
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Certificate of Analysis for MRA-914

Date: 22 JUL 2016 **Signature:**

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