Certificate of Analysis for MRA-1250

Plasmodium falciparum, Strain CamWT

Catalog No. MRA-1250

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain CamWT (originally referred to as RF 915) was isolated in 2010 from a 15-year-old human patient with malaria in Pursat province, western Cambodia. *P. falciparum,* strain CamWT was deposited as an artemisinin susceptible fast-clearing isolate.

Lot[1]: 63268000 **Manufacturing Date: 03FEB2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	29.3 ± 0.7 nM
Artemisinin	Report results	5.2 ± 0.1 nM
Quinine	Report results	86.2 ± 9.9 nM
Cycloguanil	Report results	365.4 ± 16.8 nM
Pyrimethamine	Report results	11710 ± 1623 nM
Sulfadoxine	Report results	344600 ± 47760 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	0.65%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
MSP2 PCR amplicon analysis[6]	~ 600-900 base pair amplicon	~ 900 base pair amplicon (Figure 2)
Level of Parasitemia		
Pre-freeze[7]	Report results	6.8%
Post-freeze[8]	> 1%	3.49%
Viability (post-freeze)[9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)		
Harpo's HTYE broth[10], 37°C and 26°C, aerobic	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose 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		
DNA Detection by PCR	None detected	None detected

[1]MRA-1250 was produced by cultivation of the deposited material 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 for 5 days. Every 1 to 2 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% hematocrit.

[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 1 day.

[3]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. Available at: https://www.mr4.org/Publications/MethodsinMalariaResearch.aspx].

[4]A detailed RSA_{0-3h} protocol is available on the Worldwide Antimalarial Resistance Network's website at http://www.wwarn.org/tools-resources/procedures/ring-stage-survival-assays-rsa-evaluate-vitro-and-ex-vivo-susceptibility.

[5]*P. falciparum*, strain CamWT was reported with a DHA RSA_{0-3h} value of 0.6% [Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." Science 347 (2015): 428-431. PubMed: 25502314.].

[6]Primer sequences and conditions for PCR are available upon request.

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

[8]Post-freeze parasitemia was determined after 1 day post infection by microscopic counts of Giemsa-stained blood smears.

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia (3.49%) at 1 day post infection.

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

Figure 1: MRA-1250 MSP2 Sequence

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ATTAAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT
CATAAACAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAAATGAAG GTTCTAATAC TAATAGGGTA GGTGCAAATG
CTCCAAAAGC TGATACTATT GCTAGTGGAA GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA
TCACAAACTA CTACTCCTAC CGCTGCTGAT ACCCCTACTG CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC
AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTAATTCAC CTTCACCACC 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 TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTGTTTTA
ATT
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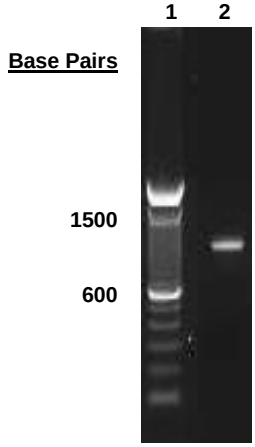
Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1250

Date: 02 DEC 2015 **Signature:**

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