b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1251

Plasmodium falciparum, Strain CamWT_C580Y

Catalog No. MRA-1251

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain CamWT_C580Y is a K13-propeller mutant of the CamWT strain (BEI Resources MRA-1250), featuring a single nucleotide substitution leading to a C580Y amino acid change. *P. falciparum*, strain CamWT_C580Y was deposited as more resistant to artemisinin than the parent strain with a ring-stage survival assay (RSA_{0-3h}) value of 8.9% when exposed to dihydroartemisinin.

Lot[1]: 63268018 **Manufacturing Date: 23JAN2015**

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	53.0 ± 6.1 nM
Artemisinin	Report results	3.0 ± 0.3 nM
Quinine	Report results	109.5 ± 15.2 nM
Cycloguanil	Report results	960.3 ± 88.6 nM
Pyrimethamine	Report results	22690 ± 2618 nM
Sulfadoxine	Report results	341100 ± 79237 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	17.24%
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	3.87%
Post-freeze[8]	> 1%	1.19%
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-1251 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 9 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 5 days.

[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

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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_C580Y was reported with a DHA RSA_{0-3h} value of 8.9% [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 9 days post infection by microscopic counts of Giemsa-stained blood smears.

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

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia (1.19%) at 5 days 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-1251 MSP2 Sequence

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TGAAATTAAA ACAACAAATT TATTTATTGA AGCAATATTA CTAGAGTTAT TTAAGAGGGA TGTTGCTGCT CCACAGTTTT
CTTTGTTACC ATCGGTACAT TCTTTTTGAC TATCAGAAGT ATTTTGTGGA TGATTATTTC TAGAACCATG CATATGTCCA
TGTTGTCCTG TACCTTTATT CTCTGGTGCA GCAGGATTTT CATTTTCTGC CGTTTGAGGT TCTTGTGGAG CTTTGGGTCC
TTCTTCAGTT GATTCATTTA ATTCATTTTG TTTTTCACTC TCTTCTCCTT TACCGTCTGT TTTATTTGGT GCATTGCCAG
AACTTGAACT TTCTGTAGTA GTGATGGGTG GTGAAGGTGA ATTACTTTCT GTAGTAGTGA TGGGTGGTGA AGGTGAATTA
CTTTCTGTAG TAGTGATGGG TGGTGAAGGT GAATTACTTT TTGTAGCAGT AGGGGTATCA GCAGCGGTAG GAGTAGTAGT
TTGTGATTCT CCATTATTAG TAGTACTAGT ACTTGCACTA TTTGTACTAC TTTGACTTCC ACTAGCAATA GTATCAGCTT
TTGGAGCATT TGCACCTACC CTATTAGTAT TAGAACCTTC ATTTGCCATA CTTCTCCTTA TACTCATATT ATAAGCATTG
TTTATGAATG TGTTGCTATA TTTACTTTCA TTTTTAATAT TAAAGGTAAC AAAAATAAA
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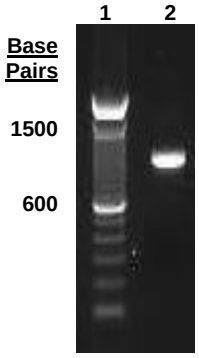
Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1251

Date: 03 DEC 2015 **Signature:**

BEI Resources Authentication

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