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]: 70018495 **Manufacturing Date: 27SEP2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2]		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[4]		
Chloroquine	Report results	45.8 ± 6.3 nM
Artemisinin	Report results	12.2 ± 0.8 nM
Quinine	Report results	68.7 ± 6.3 nM
Cycloguanil	Report results	519 ± 23.9 nM
Pyrimethamine	Report results	19740 ± 2278 nM
Sulfadoxine	Report results	409500 ± 37770 nM
Ring-stage Survival Assay (RSA_{0-3h})[5]		
Dihydroartemisinin (DHA)[6]	Report results	9.44%
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Sequencing of kelch protein (K13-propeller) gene[7] (~ 1990 base pairs)	Contains K13 C580Y mutation	Contains K13 C580Y mutation (Figure 2)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[8]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[9,10]		
Ring-stage parasitemia	Report results	3.58%
Total parasitemia	≥ 2%	5.07%
Post-freeze[2,11]		
Ring-stage parasitemia	Report results	1.26%
Total parasitemia	≥ 1%	3.78%
Viability[2,12]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[13], 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[2]		
DNA Detection by PCR	None detected	None detected

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[1]MRA-1251 was produced by cultivation of BEI Resources MR-MRA-1251 lot 62368019 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 22 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

[2]Testing completed on vialed post-freeze material

[3]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.

[4]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.beiresources.org/Publications/MethodsinMalariaResearch.aspx].

[5]A detailed $RSA_{0\text{-}3h}$ 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.

[6]*P. falciparum*, strain CamWT_C580Y was reported with a DHA $RSA_{0\text{-}3h}$ 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.].

[7]K13-propeller mutation C580Y confers artemisinin resistance *in vitro*; for additional information, please refer to Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." Science 347 (2015): 428-431. PubMed: 25502314.

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

[9]Testing completed on bulk material prior to vialing and freezing

[10]Parasitemia was determined after 22 days post infection by microscopic counts of Giemsa-stained blood smears.

[11]Parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

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

[13]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

AAACATTGTC TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA
AACAATGCTT ATAATATGAG TATAAGGAGA AGTATGGCAA ATGAAGGTTC TAATACTAAT AGGGTAGGTG CAAATGCTCC
AAAAGCTGAT ACTATTGCTA GTGGAAGTCA AAGTAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC
AAACTACTAC TCCTACCGCT GCTGATACCC CTACTGCTAC AAAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA
AGTAATTCAC CTTCACCACC CATCACTACT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG AAAGTTCAAG
TTCTGGCAAT GCACCAAATA AAACAGACGG TAAAGGAGAA GAGAGTGAAA AACAAAATGA ATTAAATGAA TCAACTGAAG
AAGGACCCAA AGCTCCACAA GAACCTCAAA CGGCAGAAAA TGAAAATCCT GCTGCACCAG AGAATAAAGG TACAGGACAA
CATGGACATA TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG ATGGTAACAA
AGAAAACTGT GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA TAAATTTGTT GTTTTAATTT
CAGCAACACT TGTTTTATCT TTTGCCATA

Figure 2: MRA-1251 K13 Sequence

GGGAACTAAT AAAGATGGGC CAAGCTGCCA TTCATTTGTA TCTGGTGAAA AGAAATGACA TGAATTTAGA ACTTCGCCAT
TTTCTCCTCC TGTAATTATA TAAGAATCTG ACAATGTGGC AGCTCCAAAA TTCATTTTTT TCTCTGGTAC ACCATTTAGA
AATTGCCATC TTTTATTAAA TGGTTGATAT TGTTCAACGG AATCTAATAT GTTATGTTCA TTATCAATAC CTCCAACAAC
ATATATTTGA TTAAGGTAAT TAAAAGCTGC TCCTGAACTT CTAGCTTCTA ATAAGGCATA TGGAAATTGT TCCCATTTAT
TCATTTTTTC TTCATATACT TCAATAGAAT TTAATCTCTC ACCATTAGTT CCACCAATGA CATAAATTTT ATTATCAAAA
GCAACATACA TAGCTGATGA TCTAGGGGTA TTCAAAGGTG CCACCTCTAC CCATGCTTTC ATACGATGAT CATATGCTTC
TACATTCGGT ATAATAGAAG AGCCATCATA TCCCCCAATA CAATAAATTC TACCATTTGA CGTAACACCA CAATTATTTC
TTCTAGGTAT ATTTAAATTA CTTGATACAT ACCAGACGTC TCTTAATCTA TCATACACCT CAGTTTCAAA TAAAGCCTTA
TAATCATAGT TATTACCACC AAAAACGTAT AAGAAATTAT TCAATACAGC ACTTCCAAAA TAAGCTTTTT TGGTAGACAT
AGGTGTACAC ATACGCCAGC ATTGTTGACT AATATCTAAT AATTCCATCG AATTTAAATA TTCTACACCA TCAAATCCAC
CTATACAAAA TACTAATGGG AATGGTAAAA ATTTAATACC ATAAAATTCT GCTTCTTTCA ACAAGGCTTC ACTTTCACTT
AAATCTTTTG GTATGGGTAT AGTTAACGGA TTTCTTAAGA AGTTAAGTAT AATTCTAAAT AACTCACTAT CCCTATCTAA
GAATATTCTT CCTTGTTTAT CTCTGGTTAC ATGATGTCTT CCACTTAATA ATTTCTCTAT AAATGAATCT TTTTGTTGTG
TTAAGGTATG TCTAGATGTT TCAAAAATAG CTCCACCAAC ATTAATATCA ATCATAGTTT CAGTAGCAAT ATTTGCATCA
ACAATTTTTT TTTTATCATT CTCAAAATTT TTATATTCTT GATAATATTT TTCTTTTTCT AAATATAATA CTAATTTAAT
TTTATCGATT TCTTGTAAAA ATCTTAATCT TTCTTCATCA AATCGTTTCC TATGTTCTTC TTTTTCTTTT TTTATTTGTT
TATAACCATT AGATATATCA ATATCTAATT TCTTTCTTTC ATCATGTAAT TTCTGTTCTT CAATATTTTT ACGGTTTTCT
AATTCTTTGT ACAATCGTAC TCTTTCCATT TCTAGTTCTT TCTTATCTTT AAATAATTTA TCTTTTTCTC GAATAAAATT
CATTTGTGTC TTTTTTAACC AATTAATAAA TGTAATTCTT AAATCACCTA CCATATTTTC AAAATCACTA GCATCACTTA
ATTCCGTTTC AATAATTTTC TTTTCATAAG TATCATTTAC ATTATTTACA GTTGAAAATT TTTTTAAATT GTTTAATCCA

GAATCATCAT TTATAAGATT TGCTGCATCT AAAAAATTCT CTTTTTTGTT GGTATTCATA ATTGATGGAG AATTCATATT
ATTATTAATA AGATTATTAG TTATATTATT TGCTGTCAGA TTATTATTAT TATTATTATT ATTATGATTA ATATTATTAT
TTTCATTTGT AATAGTATCT TTTTTTTTAT TTAACAATTT ATTTAAATAT TTATTTCCTA TATTATCTTT AGACATATTA
TTAAATGTTC TTGATAAATT ACTTGGTAAA AAATCTTTTT TACTATCAAA GTTCGAATCT AATACACTCA TATCAATGGA
TTCTAATAGG CTATCTTTAA CATTTCCATA ACTACTATTA TTTAAAAGGA AACTATTATT TTCCGTTTTC TC

/Heather Couch/
Heather Couch 29 MAY 2019
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