

***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<sub>0-3h</sub>) value of 8.9% when exposed to dihydroartemisinin. MRA-1251 lot 70054923 was produced by cultivation of the BEI Resources seed lot 63268019 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, 2 g/L D-glucose, 27 µg/mL hypoxanthine and 5 µg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia for 20 days. Every 2 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.

**Lot: 70054923**

**Manufacturing Date: 29SEP2022**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>1</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)<sup>1</sup></b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR Green I <sup>®</sup> drug sensitivity assay <sup>2</sup>		
Chloroquine	Report results	33.2 ± 3.1 nM
Artemisinin	Report results	12.2 ± 0.8 nM
Quinine	Report results	169.3 ± 15.6 nM
Cycloguanil	Report results	996.9 ± 91.9 nM
Pyrimethamine	Report results	28540 ± 1973 nM
Sulfadoxine	Report results	292500 ± 33750 nM
Ring-stage Survival Assay (RSA <sub>0-3h</sub> ) <sup>3</sup> Dihydroartemisinin (DHA)	Report results	12.99%
<b>Genotypic Analysis<sup>1</sup></b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)
<b>Level of Parasitemia by Giemsa Stain Microscopy</b> Pre-freeze (20 days post-infection) <sup>4</sup>		
Ring-stage parasitemia	Report results	3.50%
Total parasitemia	≥ 2%	5.73%
Post-freeze (4 days post-infection) <sup>1</sup>		
Ring-stage parasitemia	Report results	1.40%
Total parasitemia	≥ 1%	2.14%
<b>Viability (2 days post-infection)<sup>1</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)<sup>1</sup></b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>5</sup>	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud 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

TEST	SPECIFICATIONS	RESULTS
<b>Mycoplasma Contamination<sup>1</sup></b> DNA detection by PCR	None detected	None detected

<sup>1</sup>Testing completed on vial, post-freeze material

<sup>2</sup>A SYBR Green I<sup>®</sup> 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<sup>®</sup>-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. Methods in Malaria Research Sixth Edition is available on the [BEI Resources website](#)]

<sup>3</sup>A detailed RSA0-3h protocol is available on the [Worldwide Antimalarial Resistance Network's website](#).

<sup>4</sup>Testing completed on bulk material prior to vialing and freezing

<sup>5</sup>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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ATTAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTA CCTTAAATAT TAAAAATGAA AGTAAATATA GCAACACATT
CATAAACAAAT GCTTATAATA TGAGTATAAG GAGAAGTATG GCAAATGAAG GTTCTAATAC TAATAGGGTA GGTGCAAATG
CTCCAAAAGC TGATACTATT GCTAGTGGAA GTCAAAAGTAG 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 AAAATGAAA TCCTGCTGCA CCAGAGAATA AAGGTACAGG
ACAACATGGA CATATGCATG GTTCTAGAAA TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT ACCGATGGTA
ACAAAGAAAA CTGTGGAGCA GCAACATCCC TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTGTGTTTA
ATTTTCAGCA CACTTGTTTT ATCTTTTGC
    
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