b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

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 human patient with malaria in Pursat province, western Cambodia. *P. falciparum*, strain CamWT was deposited as an artemisinin susceptible fast-clearing isolate. MRA-1250 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per 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 for 13 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.

Lot: 70038872 **Manufacturing Date: 06OCT2020**

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[2]		
Chloroquine	Report results	72.7 ± 16.9 nM
Artemisinin	Report results	11.7 ± 1.9 nM
Quinine	Report results	133.2 ± 18.5 nM
Cycloguanil	Report results	440.0 ± 61.0 nM
Pyrimethamine	Report results	10570 ± 486.9 nM
Sulfadoxine	Report results	233500 ± 10756.9 nM
Ring-stage Survival Assay (RSA_{0-3h})		
Dihydroartemisin (DHA)[3]	Report results	0.91%
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Functional Activity by PCR Amplification[1]		
MSP2 PCR amplicon analysis	600 to 900 base pair amplicon	800 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (13 days post-infection)[4]		
Ring-stage parasitemia	Report results	3.54%
Total parasitemia	≥ 2%	5.14%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	7.26%
Total parasitemia	≥ 1%	8.33%
Viability (post-freeze; 2 days post-infection)[1]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[5]	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

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Certificate of Analysis for MRA-1250

TEST	SPECIFICATIONS	RESULTS
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination[1]		
DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material
[2]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (greater than 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.]
[3]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. *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.].
[4]Testing completed on bulk material prior to vialing and freezing
[5]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

```
TTAAAACATT GTCTATTATA AATTTCTTTA TTTTTGTTAC CTTTAATATT AAAAATGAAA GTAAATATAG CAACACATTC
ATAAACAATG CTTATAATAT GAGTATAAGG AGAAGTATGG CAAATGAAGG TTCTAATACT AATAGGGTAG GTGCAAATGC
TCCAAAAGCT GATACTATTG CTAGTGGAAG TCAAAGTAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT
CACAAACTAC TACTCCTACC GCTGCTGATA CCCCTACTGC TACAAAAAGT AATTCACCTT CACCACCCAT CACTACTACA
GAAAGTAATT CACCTTCACC ACCCATCACT ACTACAGAAA GTAATTCACC TTCACCACCC ATCACTACTA CAGAAAGTTC
AAGTTCTGGC AATGCACCAA ATAAAACAGA CGGTAAAGGA GAAGAGAGTG AAAAACAAAA TGAATTAAAT GAATCAACTG
AAGAAGGACC CAAAGCTCCA CAAGAACCTC AAACGGCAGA AAATGAAAAT CCTGCTGCAC CAGAGAATAA AGGTACAGGA
CAACATGGAC ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA
CAAAGAAAAC TGTGGAGCAG CAACATCCCT CTTAAATAAC TCTAGTAATA TTGCTTCAAT AAATAAATTT GTTGTTTTAA
TTTCAGCAAC ACTTGTTTTA TCTTTTGCCA TATTCATATA AA
```

/Heather Couch/
Heather Couch 28 MAY 2021
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898