b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1253

Plasmodium falciparum, Strain Cam5_rev

Catalog No. MRA-1253

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain Cam5_rev is a K13-propeller revertant mutant of the original Cam5 strain, featuring a reversion in wild-type allele I543T. The original Cam5 strain (also referred to as IPC 4912), was isolated in 2011 from a human patient with malaria in Mondulkiri province, eastern Cambodia. *P. falciparum*, strain Cam5_rev was deposited as susceptible to artemisinin.

Lot[1]: 63268023 **Manufacturing Date: 20FEB2015**

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	23.7 ± 1.6 nM
Artemisinin	Report results	6.1 ± 0.6 nM
Quinine	Report results	176.3 ± 20.3 nM
Cycloguanil	Report results	571.4 ± 119.3 nM
Pyrimethamine	Report results	19050 ± 3976.1 nM
Sulfadoxine	Report results	221700 ± 51500 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	0.29%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 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	3.25%
Post-freeze[8]	> 1%	2.48%
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-1253 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 8 days. Every 1 to 3 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

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

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 Cam5_rev was reported with a DHA RSA_{0-3h} value of 0.3% [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 8 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.

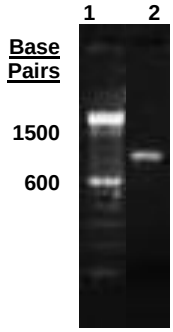
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia (2.48%) at 1 day post infection.

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

Figure 1: MRA-1253 MSP2 Sequence

```
TTTATTTATT GAAGCAATAT TACTAGAGTT ATTTAAGAGG GATGTTGCTG CTCCACAGTT TTCTTTGTTA CCATCGGTAC
ATTCTTTTTG ACTATCAGAA GTATTTTGTG GATGATTATT TCTAGAACCA TGCATATGTC CATGTTGTCC TGTACCTTTA
TTCTCTGGTG CAGCAGGATT TTCATTTTCT GCCGTTTGAG GTTCTTGTGG AGCTTTGGGT CCTTCTTCAG TTGATTCATT
TAATTCATTT TGTTTTTCAC TCTCTTCTCC TTTACCGTTT GTTTTATTTG GTGCATTGCC AGAACTTGAA CTTTCTGTAG
TAGTGATGGG TGGTGAAGGT GAATTACTTT CTGTAGTAGT GATGGGTGGT GAAGGTGAAT TACTTTCTGT AGTAGTGATG
GGTGGTGAAG GTGAATTACT TTCTGTAGTA GTGATGGGTG GTGAAGGTGA ATTACTTTTT GTAGCAGTAG GGGTATCAGC
AGCGGTAGGA GTAGTAGTTT GTGATTCTCC ATTATTAGTA GTACTAGTAC TTGCACTATT TGTACTACTT TGACTTCCAC
TAGCAATAGT ATCAGCATTT GGAGCATTTG CACCTACACT AGTAGTATTA GAACCTTCAT TTGCCATACT TCTCCTTATA
CTCATATTAT AAGCATTGTT TATGAATGTG TTGCTATATT TACTTTCATT TTTAATATTA AAGGTAAC
```

Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1253

Date: 03 DEC 2015 **Signature:**

BEI Resources Authentication

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.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

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