b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1239

Plasmodium falciparum, Strain IPC 5188

Catalog No. MRA-1239

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain IPC 5188 was isolated in 2011 from the blood of a human patient with malaria in Ratanakiri province, northeastern Cambodia. *P. falciparum*, strain IPC 5188 has shown sensitivity to artemisinin.

Lot[1]: 62401475 **Manufacturing Date: 21FEB2014**

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	13.6 ± 1.3 nM
Artemisinin	Report results	5.9 ± 1.0 nM
Quinine	Report results	302.5 ± 56.0 nM
Cycloguanil	Report results	319.6 ± 74.2 nM
Pyrimethamine	Report results	17830 ± 2057.3 nM
Sulfadoxine	Report results	342400 ± 55427.6 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisinin (DHA)[5]	Report results	0.18%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 640 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
Level of Parasitemia		
Pre-freeze[7]		
Ring-stage parasitemia	Report results	2.60%
Total parasitemia	≥ 2%	3.72%
Post-freeze[8]		
Ring-stage parasitemia	Report results	0.41%
Total parasitemia	≥ 1%	1.23%
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-1239 was produced by cultivation of the deposited material 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 23 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for 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 3 days.

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

[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 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].
[4]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.
[5]*P. falciparum*, strain IPC 5188 was deposited in 2013 with a DHA $RSA_{0\text{-}3h}$ value of 0.1%.
[6]Primer sequences and conditions for PCR are available upon request.
[7]Pre-freeze parasitemia was determined after 23 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 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-1239 MSP2 Sequence

```
AACAACAAAT TTATTTATTG AAGCAATATT ACTAGAGTTA TTTAAGAGGG ATGTTGCTGC TCCACAGTTT TCTTTGTTAC
CATCGGTACA TTCTTTTTGA CTATCAGAAG TATTTTGTGG ATGATTATTT CTAGAACCAT GCATATGTCC ATGTTGTCCT
GTACCTTTAT TCTCTGGTGC AGATTGTAAT TCGGGGGATT CAGTTTGTTC GGCTGTTGGA GCAGAATTTT CAGCTTGTTC
AGGTTGTGCA GTAGGACTTT TAGTGTCTGC ATCTTGAGTG GGTGGAACAT TTGATTTAGT TTGAGAGTCT TGTTGAACAT
TTGAGTTATT TTGAGTTTCT TTATTTGCTT GATTTGGTTC TTGAACTCCT CCATTACCTT TTGGATTTGT TTTGGCATTA
TTATGATTTG GATTTTCTGA AGAGGTACTG GTAGATGCTT CTGCATCATT AGTAGTTGTG GTAGTTGTGG TAGTTGTGGT
AGTAGCGGGA GTACTTGAAC TTCCCTCAGC ATCTGCACCA GGATTAGCAC CATTACCAGC ACTAGCAACA GCACCMGAAC
CAGCACTAGC AACAGCACCA GAACCAGCAC TACCACTAGC ACCAGTAGGA GTCTTACTTT CTGCCATACT TCTCCTTAT
```

Date: 30 APR 2017 **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.