b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for MRA-1240

## *Plasmodium falciparum*, Strain IPC 5202

## Catalog No. MRA-1240

**Product Description:** *Plasmodium falciparum* (*P. falciparum*), strain IPC 5202 was isolated in 2011 from a human patient with malaria in Battambang province, western Cambodia. *P. falciparum,* strain IPC 5202 has shown resistance to artemisinin.

**Lot[1]: 63171570** **Manufacturing Date: 14JAN2015**

| 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 | 33.7 ± 4.7 nM |
| Artemisinin | Report results | 9.9 ± 0.9 nM |
| Quinine | Report results | 193.6 ± 26.8 nM |
| Cycloguanil | Report results | 953.7 ± 132.2 nM |
| Pyrimethamine | Report results | 18590 ± 3880.1 nM |
| Sulfadoxine | Report results | 469000 ± 32423 nM |
| Ring-stage Survival Assay ($RSA_{0-3h}$)[4] | | |
| Dihydroartemisin (DHA)[5] | Report results | 68.8% |
| **Genotypic Analysis** | | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 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 | 7.27% |
| Post-freeze[8] | > 1% | 6.91% |
| **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-1240 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 9 days. Every 1 to 2 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 4 days.

[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.mr4.org/Publications/MethodsinMalariaResearch.aspx].

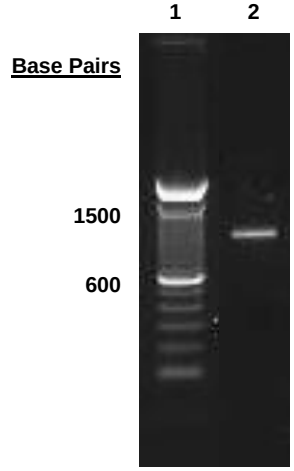
**BEI Resources**
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[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 IPC 5202 was deposited in 2013 with a DHA $RSA_{0-3h}$ value of 88.2%.
[6]Primer sequences and conditions for PCR are available upon request.
[7]Pre-freeze parasitemia was determined after 9 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia (6.91%) at 4 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-1240 MSP2 Sequence**

TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AATATGAGTA TAAGGAGAAG TATGGAAGAA AGTAATCCTT
CTACTRGTGC TGGTGGTAGT GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTGG TAGTGGTAGT
GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTTC
TGGTGATGGT AATGGTGCTA ATCCTGGTGC AGATGCTGAG AGAAGTCCAA GTACTCCCGC TACTACCACA ACTACCACAA
CTACTAATGA TGCAGAAGCA TCTACCAGTA CCTCTTCAGA AAATCCAAAT CATAATAATG CCGAAACAAA TCCAAAAGGT
AAAGGAGAAG TTCAAAAACC AAATCAAGCA AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT CTCAAACTAA
ATCAAATGTT CCACCCACTC AAGATGCAGA CACTAAAAGT CSTACTGCAC AACCTGAACA AGCTGAAAAT TCTGCTCCAA
CARCCGAACA AACTGAATCC CCCGAATTAC MTSTGCACCA GAGAATAAAG GTACAGGMCA ACATGGACAT ATGCATGGTT
CTAGAAATAA TCATCCACAA AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGAAAACTG TGGAGCAGCA
ACATCCCTCT TAAGTAACTC TAGTAATAT

**Figure 2: PCR Amplification of MSP2**




**Lane 1: 100 base pair ladder**
**Lane 2: 100 ng of MRA-1240**

**Date:** 02 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.



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