

***Plasmodium falciparum*, Strain IPC 4912**

**Catalog No. MRA-1241**

**Product Description:** *Plasmodium falciparum* (*P. falciparum*), strain IPC 4912 was isolated in 2011 from the blood of a human patient with malaria in Mondulkiri province, southeastern Cambodia. *P. falciparum*, strain IPC 4912 has shown resistance to artemisinin.

**Lot<sup>1</sup>: 62401480**

**Manufacturing Date: 10APR2014**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>2</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)</b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR green I <sup>®</sup> drug sensitivity assay <sup>3</sup> Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine Ring-stage Survival Assay (RSA <sub>0-3h</sub> ) <sup>4</sup> Dihydroartemisinin (DHA) <sup>5</sup>	Report results Report results Report results Report results Report results Report results Report results	28.2 ± 1.3 nM 3.6 ± 0.2 nM 84.7 ± 5.9 nM 198.4 ± 55.5 nM 24490 ± 2258.8 nM 454500 ± 52441.9 nM
<b>Genotypic Analysis</b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (715 base pairs) MSP2 PCR amplicon analysis <sup>6</sup>	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon	Consistent with <i>P. falciparum</i> (Figure 1) ~ 900 base pair amplicon
<b>Level of Parasitemia</b> Pre-freeze <sup>7</sup> Ring-stage parasitemia Total parasitemia Post-freeze <sup>8</sup> Ring-stage parasitemia Total parasitemia	Report results ≥ 2%  Report results ≥ 1%	3.91% 5.96%  2.39% 4.34%
<b>Viability (post-freeze)<sup>9</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>10</sup> , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
<b>Mycoplasma Contamination</b> DNA Detection by PCR	None detected	None detected

<sup>1</sup>MRA-1241 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<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia daily for 27 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.

<sup>2</sup>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.

<sup>3</sup>A SYBR Green I<sup>®</sup> anti-malarial drug sensitivity assay in 96-well plates was used to determine IC<sub>50</sub> 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. Available at: <https://www.beiresources.org/Publications/MethodsInMalariaResearch.aspx>].

<sup>4</sup>A detailed RSA<sub>0-3h</sub> 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>.

<sup>5</sup>*P. falciparum*, strain IPC 4912 was deposited in 2013 with a DHA RSA<sub>0-3h</sub> value of 49.3%.

<sup>6</sup>Primer sequences and conditions for PCR are available upon request.

<sup>7</sup>Pre-freeze parasitemia was determined after 27 days post infection by microscopic counts of Giemsa-stained blood smears.

<sup>8</sup>Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.

<sup>9</sup>Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.

<sup>10</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Figure 1: MRA-1241 MSP2 Sequence**

```
TTTTTGTTAC CTTTAATATT AAAAATGAAA GTAAATATAG CAACACATTC ATAAACAATG CTTATAATAT GAGTATAAGG
AGAAGTATGG CAAATGAAGG TTCTAATACT ACTAGTGTAG GTGCAAATGC TCCAAATGCT GATACTATTG CTAGTGGAAAG
TCAAAGTAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT CACAACTAC TACTCCTACC GCTGCTGATA
CCCCTACTGC TACAAAAAGT AATTCACCTT CACCACCCAT CACTACTACA GAAAGTAATT CACCTTCACC ACCCATCACT
ACTACAGAAA GTAATTCACC TTCACCACCC ATCACTACTA CAGAAAGTAA TTCACCTTCA CCACCCATCA CTACTACAGA
AAGTTCAAGT TCTGGCAATG CACCAAATAA AACAGACGGT AAAGGAGAAG AGAGTGAAAA ACAAATGAA TTAAATGAAT
CAACTGAAGA AGGACCCAAA GCTCCACAAG AACCTCAAAC GGCAGAAAAAT GAAAATCCTG CTGCACCAGA GAATAAAGGT
ACAGGACAAC ATGGACATAT GCATGGTTCT AGAAATAATC ATCCACAAAA TACTTCTGAT AGTCAAAAAG AATGTACCGA
TGGTAACAAA GAAAACGTGT GAGCAGCAAC ATCCCTCTTA AATAACTCTA GTAATATTGC TTCAATAAAT AAATT
```

**Date:** 30 APR 2017

**Signature:**



BEI Resources Authentication

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

ATCC<sup>®</sup> 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.

