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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for MRA-1241

## *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[1]: 63171572** **Manufacturing Date: 18JAN2015**

| 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 | 37.1 ± 2.6 nM |
| Artemisinin | Report results | 9.3 ± 1.5 nM |
| Quinine | Report results | 259.6 ± 30.0 nM |
| Cycloguanil | Report results | 334.8 ± 77.8 nM |
| Pyrimethamine | Report results | 13950 ± 4914.5 nM |
| Sulfadoxine | Report results | 103400 ± 49318 nM |
| Ring-stage Survival Assay ($RSA_{0-3h}$)[4] | | |
| Dihydroartemisin (DHA)[5] | Report results | 43.2% |
| **Genotypic Analysis** | | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 730 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] | Report results | 6.5% |
| Post-freeze[8] | > 1% | 4.42% |
| **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-1241 was produced by cultivation of MR-MRA-1241 lot 62401484 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 10 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.beiresources.org/Publications/MethodsinMalariaResearch.aspx].

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[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 4912 was deposited in 2013 with a DHA $RSA_{0-3h}$ value of 49.3%.
[6]Primer sequences and conditions for PCR are available upon request.
[7]Pre-freeze parasitemia was determined after 10 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 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-1241 MSP2 Sequence**

ACAACAAATT TATTTATTGA AGCAATATTA CTAGAGTTAT TTAAGAGGGA TGTTGCTGCT CCACAGTTTT CTTTGTTACC
ATCGGTACAT TCTTTTTGAC TATCAGAAGT ATTTTGTGGA TGATTATTTC TAGAACCATG CATATGTCCA TGTTGTCCTG
TACCTTTATT CTCTGGTGCA GCAGGATTTT CATTTTCTGC CGTTTGAGGT TCTTGTGGAG CTTTGGGTCC TTCTTCAGTT
GATTCATTTA ATTCATTTTG TTTTTCACTC TCTTCTCCTT TACCGTCTGT TTTATTTGGT GCATTGCCAG AACTTGAACT
TTCTGTAGTA GTGATGGGTG GTGAAGGTGA ATTACTTTCT GTAGTAGTGA TGGGTGGTGA AGGTGAATTA CTTTCTGTAG
TAGTGATGGG TGGTGAAGGT GAATTACTTT CTGTAGTAGT GATGGGTGGT GAAGGTGAAT TACTTTTTGT AGCAGTAGGG
GTATCAGCAG CGGTAGGAGT AGTAGTTTGT GATTCTCCAT TATTAGTAGT ACTAGTACTT GCACTATTTG TACTACTTTG
ACTTCCACTA GCAATAGTAT CAGCATTTGG AGCATTTGCA CCTACACTAG TAGTATTAGA ACCTTCATTT GCCATACTTC
TCCTTATACT CATATTATAA GCATTGTTTA TGAATGTGTT GCTATATTTA CTTTCATTTT TAATATTAAA GGTAACAAAA
ATAAAGAA

**Date:** 01 JUN 2016 **Signature:**

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