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]: 64417019 **Manufacturing Date: 22JUL2016**

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	63.0 ± 4.4 nM
Artemisinin	Report results	4.1 ± 0.5 nM
Quinine	Report results	349.7 ± 24.2 nM
Cycloguanil	Report results	351.1 ± 81.6 nM
Pyrimethamine	Report results	26740 ± 4328.7 nM
Sulfadoxine	Report results	226700 ± 47317 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	29.3%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 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
Sequencing of kelch protein (K13-propeller) gene[7] (~ 1970 base pairs)	K13 I543T mutation present	K13 I543T mutation present (Figure 2)
Level of Parasitemia		
Pre-freeze[8]	Report results	4.47%
Post-freeze[9]	> 1%	9.03%
Viability (post-freeze)[10]	Growth in infected red blood cells	Growth in infected red blood cells (Figure 3)
Sterility (21-day incubation)		
Harpo's HTYE broth[11], 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 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 6 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

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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_{0-3h} 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]K13-propeller mutation I543T confers artemisinin resistance *in vitro*; for additional information, please refer to Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." Science 347 (2015): 428-431. PubMed: 25502314.

[8]Pre-freeze parasitemia was determined after 8 days post infection by microscopic counts of Giemsa-stained blood smears.

[9]Post-freeze parasitemia was determined after 6 days post infection by microscopic counts of Giemsa-stained blood smears.

[10]Viability was confirmed by examination of infected erythrocytes for parasitemia at 6 days post infection.

[11]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

TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA CTAGTGTAGG TGCAAATGCT
CCAAATGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAAACTACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAAAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG
AAAGTAATTC ACCTTCACCA CCCATCACTA CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTAAT
TCACCTTCAC CACCCATCAC TACTACAGAA AGTTCAAGTT CTGGCAATGC ACCAAATAAA ACAGACGGTA AAGGAGAAGA
GAGTGAAAAA CAAAATGAAT TAAATGAATC AACTGAAGAA GGACCCAAAG CTCCACAAGA ACCTCAAACG GCAGAAAATG
AAAATCCTGC TGCACCAGAG AATAAAGGTA CAGGACAACA TGGACATATG CATGGTTCTA GAAATAATCA TCCACAAAAT
ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG AAAACTGTGG AGCAGCAACA TCCCTCTTAA ATAACTCTAG
TAATATTGCT TCAATAAATA AATTTGTTGT TTTAATT

Figure 2: MRA-1241 K13 Sequence

CCATTCATTT GTATCTGGTG AAAAGAAATG ACATGAATTT AGAACTTCGC CATTTTCTCC TCCTGTAATT ATATAAGAAT
CTGACAATGT GGCAGCTCCA AAATTCATTT TTTTCTCTGG TACACCATTT AGAAATTGCC ATCTTTTATT AAATGGTTGA
TATTGTTCAA CGGAATCTAA TATGTTATGT TCATTATCAA TACCTCCAAC AACATATATT TGATTAAGGT AATTAAAAGC
TGCTCCTGAA CTTCTAGCTT CTAATAAGGC ATATGGAAAT TGTTCCCATT TATTCATTTT TTCTTCATAT ACTTCAATAG
AATTTAATCT CTCACCATTA GTTCCACCAA TGACATAAAT TTTATTATCA AAAGCAACAC ACATAGCTGA TGATCTAGGG
GTATTCAAAG GTGCCACCTC TACCCATGCT TTCATACGAT GATCATATGC TTCTACATTC GGTATAATAG AAGAGCCATC
ATATCCCCCA GTACAATAAA TTCTACCATT TGACGTAACA CCACAATTAT TTCTTCTAGG TATATTTAAA TTACTTGAAA
CATACCATAC ATCTCTTAAA CGATCATACA CCTCAGTTTC AAATAAAGCC TTATAATCAT AGTTATTACC ACCAAAAACG
TATAAGAAAT TATTCAATAC AGCACTTCCA AAATAAGCTT TTTTGGTAGA CATAGGTGTA CACATACGCC AGCATTGTTG
ACTAATATCT AATAATTCCA TCGAATTTAA ATATTCTACA CCATCAAATC CACCTATACA AAATACTAAT GGGAATGGTA
AAAATTTAAT ACCATAAAAT TCTGCTTCTT TCAACAAGGC TTCACTTTCA CTTAAATCTT TTGGTATGGG TATAGTTAAC
GGATTTCTTA AGAAGTTAAG TATAATTCTA AATAACTCAC TATCCCTATC TAAGAATATT CTTCCTTGTT TATCTCTGGT
TACATGATGT CTTCCACTTA ATAATTTCTC TATAAATGAA TCTTTTTGTT GTGTTAAGGT ATGTCTAGAT GTTTCAAAAA
TAGCTCCACC AACATTAATA TCAATCATAG TTTCAGTAGC AATATTTGCA TCAACAATTT TTTTTTTTAT CATTCTCAAA
ATTTTTATAT TCTTGATAAT ATTTTTCTTT TTCTAAATAT AATACTAATT TAATTTTATC GATTTCTTGT AAAAATCTTA
ATCTTTCTTC ATCAAATCGT TTCCTATGTT CTTCTTTTTC TTTTTTTATT TGTTTATAAC CATTAGATAT ATCAATATCT
AATTTCTTTC TTTCATCATG TAATTTCTGT TCTTCAATAT TTTTACGGTT TTCTAATTCT TTGTACAATC GTACTCTTTC
CATTTCTAGT TCTTTCTTAT CTTTAAATAA TTTATCTTTT TCTCGAATAA AATTCATTTG TGTCTTTTTT AACCAATTAA
TAAATGTAAT TCTTAAATCA CCTACCATAT TTTCAAAATC ACTAGCATCA CTTAATTCCG TTTCAATAAT TTTCTTTTCA
TAAGTATCAT TTACATTATT TACAGTTGAA AATTTTTTTA AATTGTTTAA TCCAGAATCA TCATTTATAA GATTTGCTGC
ATCTAAAAAA TTCTCTTTTT TGTTGGTATT CATAATTGAT GGAGAATTCA TATTATTATT AATAAGATTA TTAGTTATAT
TATTTGCTGT CAGATTATTA TTATTATTAT TATTATTATG ATTAATATTA TTATTTTCAT TTGTAATAGT ATCTTTTTTT
TTATTTAACA ATTTATTTAA ATATTTATTT CCTATATTAT CTTTAGACAT ATTATTAAAT GTTCTTGATA AATTACTTGG
TAAAAAATCT TTTTTACTAT CAAAGTTCGA ATCTAATACA CTCATATCAA TGGATTCTAA TAGGCTATCT TTAACATTTC
CATAACTACT ATTATTTAAA AGGAAACTAT TATTTTCCGT TTTCTC

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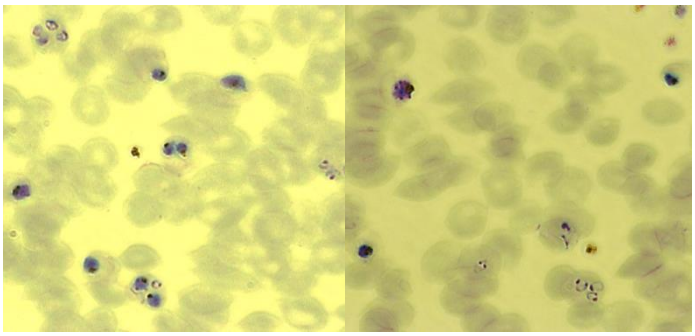
Figure 3: Viability of *P. falciparum*, Strain IPC 4912



Date: 09 FEB 2017 **Signature:**

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