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]: 64417018 **Manufacturing Date: 25JUL2016**

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	54.5 ± 3.8 nM
Artemisinin	Report results	6.4 ± 0.4 nM
Quinine	Report results	268.8 ± 37.3 nM
Cycloguanil	Report results	952.7 ± 109.9 nM
Pyrimethamine	Report results	31690 ± 2922.9 nM
Sulfadoxine	Report results	429800 ± 59568.1 nM
Ring-stage Survival Assay ($RSA_{0\text{-}3h}$)[4]		
Dihydroartemisin (DHA)[5]	Report results	70.7%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 850 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] (~ 1850 base pairs)	Contains K13 R539T mutation	Contains K13 R539T mutation (Figure 2)
Level of Parasitemia		
Pre-freeze[8]		
Ring-stage parasitemia	Report results	3.01%
Total parasitemia	≥ 2%	6.28%
Post-freeze[9]		
Ring-stage parasitemia	Report results	1.01%
Total parasitemia	≥ 1%	3.63%
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-1240 was produced by cultivation of MR-MRA-1240 lot 62401486 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 11 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

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[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].
[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]K13-propeller mutation R539T 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 11 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[10]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[11]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

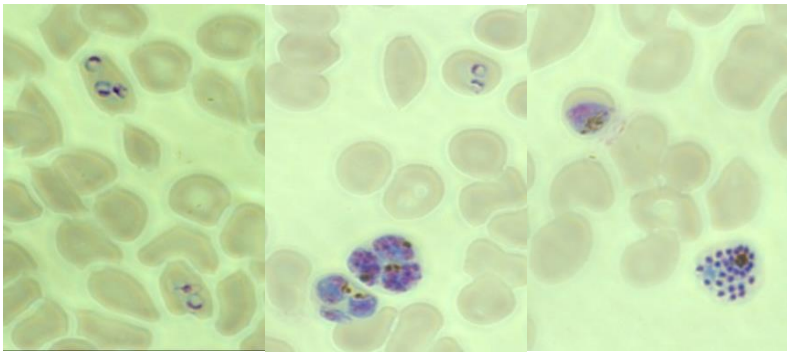
Figure 1: MRA-1240 MSP2 Sequence

AAAAATGAAA GTAAATATAG CAACACATTC ATAAACAATG CTTATAATAT GAGTATAAGG AGAAGTATGG AAGAAAGTAA
TCCTTCTACT GGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG
GTAGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT
GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTT CTGGTGATGG TAATGGTGCT AATCCTGGTG CAGATGCTGA
GAGAAGTCCA AGTACTCCCG CTACTACCAC AACTACCACA ACTACTAATG ATGCAGAAGC ATCTACCAGT ACCTCTTCAG
AAAATCCAAA TCATAATAAT GCCGAAACAA ATCCAAAAGG TAAAGGAGAA GTTCAAAAAC CAAATCAAGC AAATAAAGAA
ACTCAAAATA ACTCAAATGT TCAACAAGAC TCTCAAACTA AATCAAATGT TCCACCCACT CAAGATGCAG ACACTAAAAG
TCCTACTGCA CAACCTGAAC AAGCTGAAAA TTCTGCTCCA ACAGCCGAAC AAACTGAATC CCCCGAATTA CAATCTGCAC
CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA
AAAGAATGTA CCGATGGTAA CAAAGAAAAC TGTGGAGCAG CAACATCCCT CTTAAGTAAC TCTAGTAATA TTGCTTCAAT
AAATAAATTT GTTGTT

Figure 2: MRA-1240 K13 Sequence

ATTCATTTGT ATCTGGTGAA AAGAAATGAC ATGAATTTAG AACTTCGCCA TTTTCTCCTC CTGTAATTAT ATAAGAATCT
GACAATGTGG CAGCTCCAAA ATTCATTTTT TTCTCTGGTA CACCATTTAG AAATTGCCAT CTTTTATTAA ATGGTTGATA
TTGTTCAACG GAATCTAATA TGTTATGTTC ATTATCAATA CCTCCAACAA CATATATTTG ATTAAGGTAA TTAAAAGCTG
CTCCTGAACT TCTAGCTTCT AATAAGGCAT ATGGAAATTG TTCCCATTTA TTCATTTTTT CTTCATATAC TTCAATAGAA
TTTAATCTCT CACCATTAGT TCCACCAATG ACATAAATTT TATTATCAAA AGCAACACAC ATAGCTGATG ATCTAGGGGT
ATTCAAAGGT GCCACCTCTA CCCATGCTTT CATACGATGA TCATATGCTT CTACATTCGG TATAATAGAA GAGCCATCAT
ATCCCCCAAT ACAATAAATT GTACCATTTG ACGTAACACC ACAATTATTT CTTCTAGGTA TATTTAAATT ACTTGAAACA
TACCATACAT CTCTTAAACG ATCATACACC TCAGTTTCAA ATAAAGCCTT ATAATCATAG TTATTACCAC CAAAAACGTA
TAAGAAATTA TTCAATACAG CACTTCCAAA ATAAGCTTTT TTGGTAGACA TAGGTGTACA CATACGCCAG CATTGTTGAC
TAATATCTAA TAATTCCATC GAATTTAAAT ATTCTACACC ATCAAATCCA CCTATACAAA ATACTAATGG GAATGGTAAA
AATTTAATAC CATAAAATTC TGCTTCTTTC AACAAGGCTT CACTTTCACT TAAATCTTTT GGTATGGGTA TAGTTAACGG
ATTTCTTAAG AAGTTAAGTA TAATTCTAAA TAACTCACTA TCCCTATCTA AGAATATTCT TCCTTGTTTA TCTCTGGTTA
CATGATGTCT TCCACTTAAT AATTTCTCTA TAAATGAATC TTTTTGTTGT GTTAAGGTAT GTCTAGATGT TTCAAAAATA
GCTCCACCAA CATTAATATC AATCATAGTT TCAGTAGCAA TATTTGCATC AACAATTTTT TTTTTATCAT TCTCAAAATT
TTTATATTCT TGATAATATT TTTCTTTTTC TAAATATAAT ACTAATTTAA TTTTATCGAT TTCTTGTAAA AATCTTAATC
TTTCTTCATC AAATCGTTTC CTATGTTCTT CTTTTTCTTT TTTTATTTGT TTATAACCAT TAGATATATC AATATCTAAT
TTCTTTCTTT CATCATGTAA TTTCTGTTCT TCAATATTTT TACGGTTTTC TAATTCTTTG TACAATCGTA CTCTTTCCAT
TTCTAGTTCT TTCTTATCTT TAAATAATTT ATCTTTTTCT CGAATAAAAT TCATTTGTGT CTTTTTTAAC CAATTAATAA
ATGTAATTCT TAAATCACCT ACCATATTTT CAAAATCACT AGCATCACTT AATTCCGTTT CAATAATTTT CTTTTCATAA
GTATCATTTA CATTATTTAC AGTTGAAAAT TTTTTTAAAT TGTTTAATCC AGAATCATCA TTTATAAGAT TTGCTGCATC
TAAAAAATTC TCTTTTTTGT TGGTATTCAT AATTGATGGA GAATTCATAT TATTATTAAT AAGATTATTA GTTATATTAT
TTGCTGTCAG ATTATTATTA TTATTATTAT TATTATGATT AATATTATTA TTTTCATTTG TAATAGTATC TTTTTTTTTA
TTTAACAATT TATTTAAATA TTTATTTCCT ATATTATCTT TAGACATATT ATTAAATGTT CTTGATAAAT TACTTGGTAA
AAAAT

Figure 3: Viability (post-freeze)



Date: 18 APR 2017 **Signature:**

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