

**Plasmodium falciparum, Strain IPC 3445**

**Catalog No. MRA-1236**

**Product Description:** *Plasmodium falciparum* (*P. falciparum*), strain IPC 3445 was isolated in 2010 from the blood of a human patient with malaria in Pailin province, western Cambodia. *P. falciparum*, strain IPC 3445 has shown resistance to artemisinin.

**Lot<sup>1</sup>: 64473361**

**Manufacturing Date: 20SEP2016**

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> Dihydroartemisin (DHA) <sup>5</sup>	Report results Report results Report results Report results Report results Report results Report results	36.4 ± 5.0 nM 18.7 ± 2.6 nM 377.2 ± 34.8 nM 1114.0 ± 77.0 nM 38730 ± 1784.2 nM 290600 ± 60654 nM
<b>Genotypic Analysis</b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 700 base pairs) MSP2 PCR amplicon analysis <sup>6</sup> Sequencing of kelch protein (K13-propeller) gene <sup>7</sup> (~ 1960 base pairs)	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon Contains K13 C580Y mutation	Consistent with <i>P. falciparum</i> (Figure 1) ~ 900 base pair amplicon Contains K13 C580Y mutation (Figure 2)
<b>Level of Parasitemia</b> Pre-freeze <sup>8</sup> Post-freeze <sup>9</sup>	Report results > 1%	5.05% 4.13%
<b>Viability (post-freeze)<sup>10</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells (Figure 3)
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>11</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-1236 was produced by cultivation of MR-MRA-1236 lot 62469783 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 11 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.

<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 3445 was deposited in 2013 with a DHA RSA<sub>0-3h</sub> value of 27.3%.
- <sup>6</sup>Primer sequences and conditions for PCR are available upon request.
- <sup>7</sup>K13-propeller mutation C580Y 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.
- <sup>8</sup>Pre-freeze parasitemia was determined after 11 days post infection by microscopic counts of Giemsa-stained blood smears.
- <sup>9</sup>Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
- <sup>10</sup>Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
- <sup>11</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

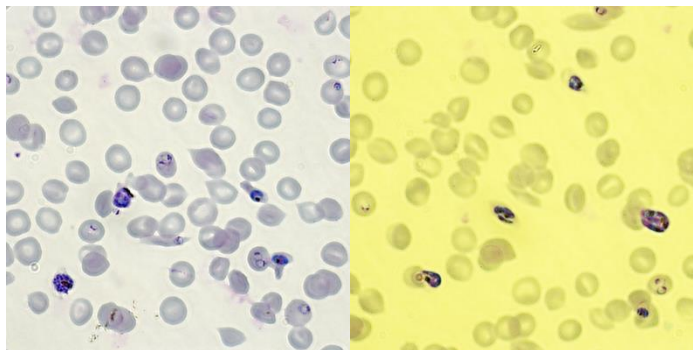
**Figure 1: MRA-1236 MSP2 Sequence**

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TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTTAAA AATGAAAAGTA AATATAGCAA CACATTCATA AACAAATGCTT
ATAATATGAG TATAAGGAGA AGTATGGCAA ATGAAGGTTT TAATACTACT AGTGTAGGTG CAAATGCTCC AAATGCTGAT
ACTATTGCTA GTGGAAGTCA AAGTAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACACTACTAC
TCCTACCGCT GCTGATACCC CTACTGCTAC AAAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTAATTCAC
CTCCACCACC CATCACTACT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG AAAGTTCAAG TTCTGGCAAT
GCACCAATAA AACACAGACGG TAAAGGAGAA GAGAGTAAAA AAAAAAATGA ATTAATGAA TCAACTGAAG AAGGACCCAA
AGCTCCACAA GAACCTCAAAA CGGCAGAAAA TGAAAAATCCT GCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA
TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT
GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA TAAATTTGTT GTT
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**Figure 2: MRA-1236 K13 Sequence**

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TTGTATCTGG TGAAAAGAAA TGACATGAAT TTAGAACTTC GCCATTTTCT CCTCCTGTAA TTATATAAGA ATCTGACAAT
GTGGCAGCTC CAAAATTCAT TTTTTTCTCT GGTACACCAT TTAGAAATTG CCATCTTTTA TTAAATGGTT GATATTGTTC
AACGGAATCT AATATGTTAT GTTCATTATC AATACCTCCA ACAACATATA TTTGATTAAG GTAATTAATA GCTGCTCCTG
AACTTCTAGC TTCTAATAAG GCATATGGAA ATTTGTTCCA TTTATTCATT TTTTCTTCAT ATACTCAAT AGAATTTAAT
CTCTCACCAT TACTTCCACC AATGACATAA ATTTTATTAT CAAAAGCAAC ATACATAGT GATGATCTAG GGGTATTCAA
AGGTGCCACC TGTACCACATG CTTTCATACG ATGATCATAT GCTTCTACAT TCGGTATAAT AGAAGAGCCA TCATATCCCC
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ACATCTCTTA AACGATCATA CACCTCAGTT TCAAATAAAG CCTTATAATC ATAGTTATTA CCACCAAAAA CGTATAAGAA
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ATACCATAAA ATTCTGCTTC TTTCAACAAG GCTTCACTTT CACTTAAATC TTTTGGTATG GGTATAGTTA ACGGATTTCT
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CCAACATTAA TATCAATCAT AGTTTCAGTA GCAATATTTG CATCAACAAT TTTTTTTTTT TCATTCTCAA AATTTTTTATA
TTCTTGATAA TATTTTTCTT TTTCTAAATA TAATACTAAT TTAATTTTAT CGATTTCTTG TAAAAATCTT AATCTTTCTT
CATCAAAATCG TTTTCTATGT TCTTCTTTTT CTTTTTTTAT TTGTTTATAA CCATTAGATA TATCAATATC TAATTTCTTT
CTTTCATCAT GTAATTTCTG TTCTTCAATA TTTTACGGT TTTCTAATTC TTTGTACAAT CGTACTCTTT CCATTTCTAG
TTCTTTCTTA TCTTTAAATA ATTTATCTTT TTCTCGAATA AAATTCATTT GTGTCTTTTT TAACCAATTA ATAAATGTAA
TTCTTAAATC ACCTACCATA TTTTCAAAAT CACTAGCATC ACTTAATTC GTTTCAATAA TTTTCTTTTC ATAAGTATCA
TTTACATTAT TTACAGTTGA AAATTTTTTT AAATTGTTTA ATCCAGAATC ATCATTTATA AGATTTGCTG CATCTAAAAA
ATTCTCTTTT TTGTTGGTAT TCATAATTGA TGGAGAATTC ATATTATTAT TAATAAGATT ATTAGTTATA TTATTTGCTG
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AATTTATTTA AATATTTTAT TCCTATATTA TCTTTAGACA TATTATTAAA TGTTCTTGAT AAATTACTTG GTAAAAAATC
TTTTTTACTA TCAAAGTTTC AATCTAATAC ACTCATATCA ATGGATTCTA ATAGGCTATC TTTAACATTT CCATAACTAC
TATTATTTAA AAGGAAACTA TTATTTTCCG TTTTCTCA
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Figure 3: Viability (post-freeze)



Date: 29 MAR 2017

Signature:



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