

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.

Lot¹: 62469782

Manufacturing Date: 28MAR2014

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>) Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ³ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine Ring-stage Survival Assay (RSA _{0-3h}) ⁴ Dihydroartemisin (DHA) ⁵	Report results Report results Report results Report results Report results Report results Report results	24.1 ± 0.6 nM 9.2 ± 0.4 nM 222.3 ± 15.4 nM 1173.0 ± 135.3 nM 28910.0 ± 1998.6 nM 332500 ± 46082.8 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 690 base pairs) MSP2 PCR amplicon analysis ⁶	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon	Consistent with <i>P. falciparum</i> (Figure 1) ~ 900 base pair amplicon
Level of Parasitemia Pre-freeze ⁷ Ring-stage parasitemia Total parasitemia Post-freeze ⁸ Ring-stage parasitemia Total parasitemia	Report results Report results Report results > 1%	3.43% 4.96% 1.37% 2.05%
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 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
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹MRA-1236 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia daily for 21 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.

²Blood-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.

³A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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>].

⁴A detailed RSA_{0-3h} protocol is available on the Worldwide Antimalarial Resistance Network's website at <http://www.wwar.org/tools-resources/procedures/ring-stage-survival-assays-rsa-evaluate-vitro-and-ex-vivo-susceptibility>.

⁵*P. falciparum*, strain IPC 3445 was deposited in 2013 with a DHA RSA_{0-3h} value of 27.3%.

⁶Primer sequences and conditions for PCR are available upon request.

⁷Pre-freeze parasitemia was determined after 21 days post infection by microscopic counts of Giemsa-stained blood smears.

⁸Post-freeze parasitemia was determined after 6 days post infection by microscopic counts of Giemsa-stained blood smears.

⁹Viability was confirmed by examination of infected erythrocytes for parasitemia at 6 days post infection.

¹⁰Atlas, 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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TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA
GAAGTATGGC AAATGAAGGT TCTAATACTA CTAGTGTAGG TGCAAATGCT CCAAATGCTG ATACTATTGC TAGTGGAAGT
CAAAGTAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC ACAAACACT ACTCCTACCG CTGCTGATAC
CCCTACTGCT ACAAAAAGTA ATTACACCTC ACCACCCATC ACTACTACAG AAAGTAATTC ACCTTCACCA CCCATCACTA
CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA AGTTCTGGCA ATGCACCAAA TAAAACAGAC
GGTAAAGGAG AAGAGAGTAA AAAAAAAAAAT GAATTAAATG AATCAACTGA AGAAGGACCC AAAGCTCCAC AAGAACCTCA
AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA
ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAAC GTGGAGCAGC AACATCCCTC
TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG TTGTTTTAA
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Date: 13 MAR 2017

Signature:



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