

***Plasmodium falciparum*, Strain IPC 4884**

Catalog No. MRA-1238

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain IPC 4884 was isolated in 2011 from the blood of a human patient with malaria in Pursat province, western Cambodia. *P. falciparum*, strain IPC 4884 has shown resistance to artemisinin.

Lot¹: 62401473

Manufacturing Date: 02APR2014

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 Dihydroartemisinin (DHA) ⁵	Report results Report results Report results Report results Report results Report results Report results	12.9 ± 0.6 nM 3.6 ± 0.2 nM 60.7 ± 4.2 nM 546.5 ± 50.4 nM 16990 ± 1174.6 nM 354700 ± 49159.7 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (685 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 ≥ 2% Report results ≥ 1%	2.51% 4.46% 1.87% 3.32%
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-1238 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 28 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.

²Blood-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.

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.warn.org/tools-resources/procedures/ring-stage-survival-assays-rsa-evaluate-vitro-and-ex-vivo-susceptibility>.

⁵*P. falciparum*, strain IPC 4884 was deposited in 2013 with a DHA RSA_{0-3h} value of 6.2%.

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

⁷Pre-freeze parasitemia was determined after 28 days post infection by microscopic counts of Giemsa-stained blood smears.

⁸Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.

⁹Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 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-1238 MSP2 Sequence

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ATTTTTGTTA CCTTTAATAT TAAAAATGAA AGTAAATATA GCAACACATT CATAAACAAT GCTTATAATA TGAGTATAAG
GAGAAGTATG GCAAATGAAG GTTCTAATAC TACTAGTGTA GGTGCAAATG CTCCAAATGC TGATACTATT GCTAGTGGAA
GTCAAAGTAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA CTACTCCTAC CGCTGCTGAT
ACCCCTACTG CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC
TACTACAGAA AGTAATTCAC CTTCAACCACC CATCACTACT ACAGAAAAGTT CAAGTTCTGG CAATGCACCA AATAAACAG
ACGGTAAAGG AGAAGAGAGT AAAAAAAAAA ATGAATTTAAA TGAATCAACT GAAGAAGGAC CCAAAGCTCC ACAAGAACCT
CAAACGGCAG AAAATGAAAA TCCTGCTGCA CCAGAGAATA AAGGTACAGG ACAACATGGA CATATGCATG GTTCTAGAAA
TAATCATCCA CAAAATACTT CTGATAGTCA AAAAGAATGT ACCGATGGTA ACAAAGAAAA CTGTGGAGCA GCAACATCCC
TCTTAAATAA CTCTAGTAAT ATTGCTTCAA TAAATAAATT TGTTG
    
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Date: 30 APR 2017

Signature: 

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