

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: 63310080

Manufacturing Date: 13MAR2015

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	25.8 ± 1.8 nM 9.5 ± 0.2 nM 110.0 ± 5.1 nM 552.8 ± 25.5 nM 23130 ± 1599 nM 398900 ± 18377 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 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) ~ 800 base pair amplicon
Level of Parasitemia Pre-freeze ⁷ Post-freeze ⁸	Report results > 1%	6.1% 3.79%
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 MR-MRA-1238 lot 62401488 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 10 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 3 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.wwarn.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%. Results are shown for MRA-1238 lot 62401473.

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

⁷Pre-freeze parasitemia was determined after 10 days post infection by microscopic counts of Giemsa-stained blood smears.

⁸Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.

⁹Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 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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TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTTAA AATGAAAGTA AATATAGCAA CACATTCATA AACAAATGCTT
ATAATATGAG TATAAGGAGA AGTATGGCAA ATGAAGGTTC TAATACTACT AGTGTAGGTG CAAATGCTCC AAATGCTGAT
ACTATTGCTA GTGGAAGTCA AAGTAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACTACTAC
TCCTACCGCT GCTGATACCC CTACTGCTAC AAAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTAATTAC
CTTCACCACC CATCACTACT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG AAAGTTCAAG TTCTGGCAAT
GCACCAAATA AAACAGACGG TAAAGGAGAA GAGAGTAAAA AAAAAAATGA ATTAAATGAA TCAACTGAAG AAGGACCCAA
AGCTCCACAA GAACCTCAAA CGGCAGAAAA TGAAAAATCCT GCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA
TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT
GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA TAAATTTGTT GTTTTAATTT CAGCAACACT
TGTTTTATCT TTTGCC
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Date: 02 JUN 2016

Signature:



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