b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1237

Plasmodium falciparum, Strain IPC 3663

Catalog No. MRA-1237

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain IPC 3663 was isolated in 2010 from the blood of a human patient with malaria in Pailin province, western Cambodia. *P. falciparum*, strain IPC 3663 has shown sensitivity to artemisinin.

Lot[1]: 62401470 **Manufacturing Date: 19FEB2014**

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	10.3 ± 0.7 nM
Artemisinin	Report results	8.2 ± 1.3 nM
Quinine	Report results	40.3 ± 3.7 nM
Cycloguanil	Report results	1305 ± 211.3 nM
Pyrimethamine	Report results	14170 ± 979.6 nM
Sulfadoxine	Report results	239200 ± 11049.6 nM
Ring-stage Survival Assay ($RSA_{0\text{-}3h}$)[4]		
Dihydroartemisinin (DHA)[5]	Report results	0.60%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 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
Level of Parasitemia		
Pre-freeze[7]		
Ring-stage parasitemia	Report results	3.27%
Total parasitemia	≥ 2%	5.78%
Post-freeze[8]		
Ring-stage parasitemia	Report results	0.50%
Total parasitemia	≥ 1%	1.24%
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	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-1237 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 20 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

[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 3 days.

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[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 $RSA_{0\text{-}3h}$ 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 3663 was deposited in 2013 with a DHA $RSA_{0\text{-}3h}$ value of 0.1%.

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

[7]Pre-freeze parasitemia was determined after 20 days post infection by microscopic counts of Giemsa-stained blood smears. This calculation is done as a fraction of infected erythrocytes in the population.

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

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 days post infection.

[10]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1237 MSP2 Sequence

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AACATTGTCT ATTATAAATT TCTTTATTTT TGTTACCTTT AATATTAAAA ATGAAAGTAA ATATAGCAAC ACATTCATAA
ACAATGCTTA TAATATGAGT ATAAGGAGAA GTATGGCAAA TGAAGGTTCT AATACTACTA GTGTAGGTGC AAATGCTCCA
AAAGCTGATA CTATTGCTAG TGGAAGTCAA AGTAGTACAA ATAGTGCAAG TACTAGTACT ACTAATAATG GAGAATCACA
AACTACTACT CCTACCGCTG CTGATACCCC TACTGCTACA AAAAGTAATT CACCTTCACC ACCCATCACT ACTACAGAAA
GTAATTCACC TTCACCACCC ATCACTACTA CAGAAAGTAA TTCACCTTCA CCACCCATCA CTACTACAGA AAGTTCAAGT
TCTGGCAATG CACCAAATAA AACAGACGGT AAAGGAGAAG AGAGTGAAAA ACAAAATGAA TTAAATGAAT CAACTGAAGA
AGGACCCAAA GCTCCACAAG AACCTCAAAC GGCAGAAAAT GAAAATCCTG CTGCACCAGA GAATAAAGGT ACAGGACAAC
ATGGACATAT GCATGGTTCT AGAAATAATC ATCCACAAAA TACTTCTGAT AGTCAAAAAG AATGTACCGA TGGTAACAAA
GAAAACTGTG GAGCAGCAAC ATCCCTCTTA AATAACTCTA GTAATATTGC TTCAATAAAT AAATTTGTTG TTTTAATTTC
AGCAACACTT GTTTTATCTT TT
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Date: 30 APR 2017 **Signature:**

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