b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1236

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[1]: 63171567 **Manufacturing Date: 14JAN2015**

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	18.1 ± 0.8 nM
Artemisinin	Report results	14.6 ± 0.3 nM
Quinine	Report results	171.8 ± 7.9 nM
Cycloguanil	Report results	1187.0 ± 54.7 nM
Pyrimethamine	Report results	47240 ± 3265.8 nM
Sulfadoxine	Report results	468700 ± 21592.1 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	19.7%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 660 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]	Report results	5.54%
Post-freeze[8]	> 1%	8.98%
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-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_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 8 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.

[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 4 days.

[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].

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[4]A 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 3445 was deposited in 2013 with a DHA RSA_{0-3h} value of 27.3%.
[6]Primer sequences and conditions for PCR are available upon request.
[7]Pre-freeze parasitemia was determined after 8 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 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-1236 MSP2 Sequence

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GAAGCAATAT TACTAGAGTT ATTTAAGAGG GATGTTGCTG CTCCACAGTT TTCTTTGTTA CCATCGGTAC ATTCTTTTTG
ACTATCAGAA GTATTTTGTG GATGATTATT TCTAGAACCA TGCATATGTC CATGTTGTCC TGTACCTTTA TTCTCTGGTG
CAGCAGGATT TTCATTTTCT GCCGTTTGAG GTTCTTGTGG AGCTTTGGGT CCTTCTTCAG TTGATTCATT TAATTCATTT
TTTTTTTTAC TCTCTTCTCC TTTACCGTYT GTTTTATTTG GTGCATTGCC AGAACTTGAA CTTTCTGTAG TAGTGATGGG
TGGTGAAGGT GAATTACTTT CTGTAGTAGT GATGGGTGGT GAAGGTGAAT TACTTTCTGT AGTAGTGATG GGTGGTGAAG
GTGAATTACT TTTTGTAGCA GTAGGGGTAT CAGCAGCGGT AGGAGTAGTA GTTTGTGATT CTCCATTATT AGTAGTACTA
GTACTTGCAC TATTTGTACT ACTTTGACTT CCACTAGCAA TAGTATCAGC ATTTGGAGCA TTTGCACCTA CACTAGTAGT
ATTAGAACCT TCATTTGCCA TACTTCTCCT TATACTCATA TTATAAGCAT TGTTTATGAA TGTGTTGCTA TATTTACTTT
CATTTTTAAT ATTAAA
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Date: 02 JUN 2016 **Signature:**

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