b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1254

Plasmodium falciparum, Strain Cam2_rev

Catalog No. MRA-1254

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain Cam2_rev is a K13-propeller revertant mutant of the original Cam2 strain, featuring a reversion in wild-type allele C508Y. The original Cam2 strain (also referred to as IPC 3445), was isolated in 2010 from a human patient with malaria in Pailin province, western Cambodia. *P. falciparum*, strain Cam2_rev was deposited as susceptible to artemisinin.

Lot[1]: 63268026 **Manufacturing Date: 23FEB2015**

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	22.2 ± 5.2 nM
Artemisinin	Report results	7.6 ± 0.7 nM
Quinine	Report results	123.5 ± 22.9 nM
Cycloguanil	Report results	930.2 ± 172.3 nM
Pyrimethamine	Report results	40130 ± 9322.1 nM
Sulfadoxine	Report results	107400 ± 24949 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	4.65%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
MSP2 PCR amplicon analysis[6]	~ 600-900 base pair amplicon	~ 800 base pair amplicon (Figure 2)
Level of Parasitemia		
Pre-freeze[7]	Report results	4.88%
Post-freeze[8]	> 1%	4.60%
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-1254 was produced by cultivation of the deposited material in fresh human erythrocytes 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 17 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

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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.mr4.org/Publications/MethodsinMalariaResearch.aspx].

[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 Cam2_rev was reported with a DHA RSA_{0-3h} value of 2.4% [Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." Science 347 (2015): 428-431. PubMed: 25502314.].

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

[7]Pre-freeze parasitemia was determined after 17 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 (4.60%) 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-1254 MSP2 Sequence

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ATGGCAAAAG ATWAAACAAG TGTTGCTGAA ATTAAAACAA CAAATTTATT TATTGAAGCA ATATTACTAG AGTTATTTAA
GAGGGATGTT GCTGCTCCAC AGTTTTCTTT GTTACCATCG GTACATTCTT TTTGACTATC AGAAGTATTT TGTGGATGAT
TATTTCTAGA ACCATGCATA TGTCCATGTT GTCCTGTACC TTTATTCTCT GGTGCAGCAG GATTTTCATT TTCTGCCGTT
TGAGGTTCTT GTGGAGCTTT GGGTCCTTCT TCAGTTGATT CATTTAATTC ATTTTTTTTT TTACTCTCTT CTCCTTTACC
GTYTGTTTTA TTTGGTGCAT TGCCAGAACT TGAACTTTCT GTAGTAGTGA TGGGTGGTGA AGGTGAATTA CTTTCTGTAG
TAGTGATGGG TGGTGAAGGT GAATTACTTT CTGTAGTAGT GATGGGTGGT GAAGGTGAAT TACTTTTTGT AGCAGTAGGG
GTATCAGCAG CGGTAGGAGT AGTAGTTTGT GATTCTCCAT TATTAGTAGT ACTAGTACTT GCACTATTTG TACTACTTTG
ACTTCCACTA GCAATAGTAT CAGCATTTGG AGCATTTGCA CCTACACTAG TAGTATTAGA ACCTTCATTT GCCATACTTC
TCCTTATACT CATATTATAA GCATTGTTTA TGAATGTGTT GCTATATTTA CTTTCATTTT TAATATTAAA GGTAACAAAA
ATAAA
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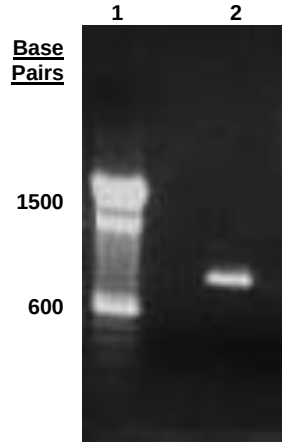
Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1254

Date: 03 DEC 2015 **Signature:**

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