Certificate of Analysis for MRA-1252

Plasmodium falciparum, Strain Cam3.I_rev

Catalog No. MRA-1252

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain Cam3.I_rev is a K13-propeller revertant mutant of the original Cam3.I strain, featuring a reversion in wild-type allele R539T. The original Cam3.I strain (also referred to as IPC 5202), was isolated in 2011 from a human patient with malaria in Battambang province, western Cambodia. *P. falciparum*, strain Cam3.I_rev was deposited as susceptible to artemisinin.

Lot[1]: 63268022 **Manufacturing Date: 25FEB2015**

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	40.1 ± 3.7 nM
Artemisinin	Report results	9.8 ± 0.7 nM
Quinine	Report results	242.7 ± 45.0 nM
Cycloguanil	Report results	997.5 ± 115.1 nM
Pyrimethamine	Report results	13960 ± 3243 nM
Sulfadoxine	Report results	126200 ± 60193 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	2.68%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 820 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 (Figure 2)
Level of Parasitemia		
Pre-freeze[7]	Report results	5.79%
Post-freeze[8]	> 1%	9.71%
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-1252 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 6 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 2 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 $RSA_{0\text{-}3h}$ 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 Cam3.I_rev was reported with a DHA $RSA_{0\text{-}3h}$ value of 0.3% [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 6 days post infection by microscopic counts of Giemsa-stained blood smears.

[8]Post-freeze parasitemia was determined after 2 days post infection by microscopic counts of Giemsa-stained blood smears.

[9]Viability was confirmed by examination of infected erythrocytes for parasitemia (9.71%) at 2 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-1252 MSP2 Sequence

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TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGA
AGAAAGTAAT CCTTCTACTG GTGCTGGTGG TAGTGGTAGT GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT GGTAGTGCTG
GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTGG TAGTGGTAGT GCTGGTGGTA GTGGTAGTGC TGGTGGTAGT
GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTTC TGGTGATGGT AATGGTGCTA ATCCTGGTGC
AGATGCTGAG AGAAGTCCAA GTACTCCCGC TACTACCACA ACTACCACAA CTACTAATGA TGCAGAAGCA TCTACCAGTA
CCTCTTCAGA AAATCCAAAT CATAATAATG CCGAAACAAA TCCAAAAGGT AAAGGAGAAG TTCAAAAACC AAATCAAGCA
AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT CTCAAACTAA ATCAAATGTT CCACCCACTC AAGATGCAGA
CACTAAAAGT CCTACTGCAC AACCTGAACA AGCTGAAAAT TCTGCTCCAA CAGCCGAACA AACTGAATCC CCCGAATTAC
AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT
GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAACT GTGGAGCAGC AACATCCCTC TTAAGTAACT CTAGTAATAT
TGCTTCAATA AATAAAT
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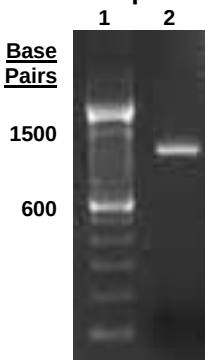
Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1252

Date: 03 DEC 2015 **Signature:**

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