Certificate of Analysis for MRA-1255

Plasmodium falciparum, Strain Dd2_R539T

Catalog No. MRA-1255

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain Dd2_R539T is a K13-propeller mutant of the Dd2 strain, featuring a single nucleotide substitution leading to a R539T amino acid change. *P. falciparum*, strain Dd2 was isolated in 1980 in Indochina. *P. falciparum*, strain Dd2_R539T was deposited as more resistant to artemisinin than the parent strain, with a ring-stage survival assay (RSA_{0-3h}) value of 19.4% when exposed to dihydroartemisinin.

Lot[1]: 63268027 **Manufacturing Date: 27JAN2015**

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	30.2 ± 3.5 nM
Artemisinin	Report results	4.1 ± 0.3 nM
Quinine	Report results	82.6 ± 11.4 nM
Cycloguanil	Report results	383.1 ± 80.0 nM
Pyrimethamine	Report results	32490 ± 3749 nM
Sulfadoxine	Report results	375600 ± 52056 nM
Ring-stage Survival Assay (RSA_{0-3h})[4]		
Dihydroartemisin (DHA)[5]	Report results	9.4%
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 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	3.9%
Post-freeze[8]	> 1%	2.65%
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-1255 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 2 days. Uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise daily 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 Dd2_R539T was reported with a DHA $RSA_{0\text{-}3h}$ value of 19.4% [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 2 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 (2.65%) 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-1255 MSP2 Sequence

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TTTATTTATT GAAGCAATAT TACTAGAGTT ATTTAAGAGG GATGTTGCTG CTCCACAGTT TTCTTTGTTA CCATCGGTAC
ATTCTTTTTG ACTATCAGAA GTATTTTGTG GATGATTATT TCTAGAACCA TGCATATGTC CATGTTGTCC TGTACCTTTA
TTCTCTGGTG CAGCAGGATT TTCATTTTCT GCCGTTTGAG GTTCTTGTGG AGCTTTGGGT CCTTCTTCAG TTGATTCATT
TAATTCATTT TGTTTTTCAC TCTCTTCTCC TTTACCGTCT GTTTTATTTG GTGCATTGCC AGAACTTGAA CTTTCTGTAG
TAGTGATGGG TGGTGAAGGT GAATTACTTT CTGTAGCAGT AGGGGTATCA GCAGCGGTAG GAGTAGTAGT TTGTGATTCT
CCATTATTAG TAGTACTAGT ACTTGCACTA TTTGTACTCC TTTGACTTCC ACTAGCAATA GTATCAGCAG CGGTAGGAGT
AGTAGTTTGT GATTCTCCAT TATTAGTAGT ACTAGTACTT GCACTATTTG TACTCCTTTG ACTTCCACTA GCAATAGTAT
CAGCAGCGGT AGGAGTAGTA GTTTGTGATT CTCCATTATT AGTAGTACTA GTACTTGCAC TATTTGTACT ACTTTGACTT
CCACTAGCAA TAGTATCAGC ATTTGGAGCA TTTGCACCTA CACTAGTAGT ATTAGAACCT TCATTTGCCA TACTTCTCCT
TATACTCATA TTATAAGCAT TGTTTATGAA TGTGTTGCTA TATTTACTTT CATTTTTAATA TTAAAGG
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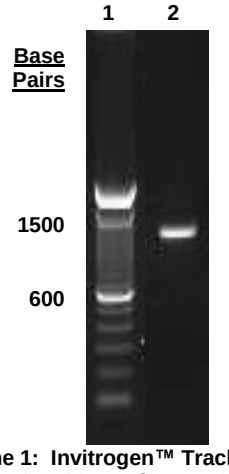
Figure 2: PCR Amplification of MSP2



Lane 1: Invitrogen™ TrackIt™ 100 bp DNA ladder
Lane 2: 100 ng of MRA-1255

Date: 03 DEC 2015 **Signature:**

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