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]: 70018497 **Manufacturing Date: 01OCT2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2]		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[4]		
Chloroquine	Report results	29.8 ± 1.4 nM
Artemisinin	Report results	11.4 ± 0.5 nM
Quinine	Report results	83.3 ± 1.0 nM
Cycloguanil	Report results	1126 ± 129.9 nM
Pyrimethamine	Report results	27610 ± 2547 nM
Sulfadoxine	Report results	419300 ± 19316 nM
Ring-stage Survival Assay (RSA_{0-3h})[5]		
Dihydroartemisinin (DHA)[6]	Report results	15.5%
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Sequencing of kelch protein (K13-propeller) gene[7] (~ 820 base pairs)	Contains K13 R539T mutation	Contains K13 R539T mutation (Figure 2)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[8]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[9,10]		
Ring-stage parasitemia	Report results	2.14%
Total parasitemia	≥ 2%	5.35%
Post-freeze[2,11]		
Ring-stage parasitemia	Report results	1.10%
Total parasitemia	≥ 1%	3.01%
Viability[2,12]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[13], 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[2]		
DNA Detection by PCR	None detected	None detected

[1]MRA-1255 was produced by cultivation of BEI Resources MR-MRA-1255 lot 63268028 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

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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 as needed and monitored for hematocrit.

[2]Testing completed on vialed post-freeze material

[3]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.

[4]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].

[5]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.

[6]*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.].

[7]K13-propeller mutation R539T confers artemisinin resistance *in vitro*; for additional information, please refer to Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." Science. 347 (2015): 428-431. PubMed: 25502314.

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

[9]Testing completed on bulk material prior to vialing and freezing

[10]Parasitemia was determined after 17 days post infection by microscopic counts of Giemsa-stained blood smears.

[11]Parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

[12]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.

[13]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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AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT
TCTAATACTA CTAGTGTAGG TGCAAATGCT CCAAATGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA CAAATAGTGC
AAGTACTAGT ACTACTAATA ATGGAGAATC ACAAACTACT ACTCCTACCG CTGCTGATAC TATTGCTAGT GGAAGTCAAA
GGAGTACAAA TAGTGCAAGT ACTAGTACTA CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC TGATACTATT
GCTAGTGGAA GTCAAAGGAG TACAAATAGT GCAAGTACTA GTACTACTAA TAATGGAGAA TCACAAACTA CTACTCCTAC
CGCTGCTGAT ACCCCTACTG CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA AGTTCTGGCA
ATGCACCAAA TAAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA AGAAGGACCC
AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA
TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAACT
GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTC
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Figure 2: MRA-1255 K13 Sequence

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TGTATAGGTG GATTTGATGG TGTAGAATAT TTAAATTCGA TGGAATTATT AGATATTAGT CAACAATGCT GGCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT
ATGATTATAA GGCTTTATTT GAAACAGAGG TGTATGATAG ATTAAGAGAC GTCTGGTATG TATCAAGTAA TTTAAATATA
CCTAGAAGAA ATAATTGTGG TGTTACGTCA AATGGTACAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC
GAATGTAGAA GCATATGATC ATCGTATGAA AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT
GTGTTGCTTT TGATAATAAA ATTTATGTCA TTGGTGGAAC TAATGGTGAG AGATTAAATT CTATTGAAGT ATATGAAGAA
AAAATGAATA AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG TTCAGGAGCA GCTTTTAATT ACCTTAATCA
AATATATGTT GTTGGAGGTA TTGATAATGA ACATAACATA TTAGATTCCG TTGAACAATA TCAACCATTT AATAAAAGAT
GGCAATTTCT AAATGGTGTA CCAGAGAAAA AAATGAATTT TGGAGCTGCC ACATTGTCAG ATTCTTATAT AATTACAGGA
GGAGAAAATG GCGAAGTTCT AAATTCATGT CATTTCTTTT CACCAGATAC AAATGAATGG CAGCTTGGCC CATCTTTATT
AGTTCCCAGA TTTGGTCACT CCG
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E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898