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 revertant 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. MRA-1255 lot 70068225 was produced by cultivation of the BEI Resources seed 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, 2 g/L D-glucose, 27 µg/mL hypoxanthine and 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 for 19 days. Every 2 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.

Lot: 70068225 **Manufacturing Date: 28MAY2024**

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TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[1]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[1]		
Half-maximal Inhibitory Concentration (IC_{50}) by SYBR Green I® drug sensitivity assay[2]		
Chloroquine	Report results	38.7 ± 0.9 nM
Artemisinin	Report results	19.0 ± 0.4 nM
Quinine	Report results	103.2 ± 9.5 nM
Cycloguanil	Report results	950.0 ± 43.8 nM
Pyrimethamine	Report results	22310 ± 1542 nM
Sulfadoxine	Report results	433100 ± 29941 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 830 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Sequencing of kelch protein (K13-propeller) gene (~ 2100 base pairs)[3]	Contains K13 R539T mutation	Contains K13 R539T mutation (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (19 days post-infection)[4]		
Ring-stage parasitemia	Report results	3.09%
Total parasitemia	≥ 2%	4.38%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.21%
Total parasitemia	≥ 1%	1.26%
Viability (2 days post-infection)[1]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (14-day incubation)[1]		
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°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[1]		
DNA detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material

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[2]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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]

[3]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.

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

Figure 1: MRA-1255 MSP2 Sequence

TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAATGCTT ATAATATGAG
TATAAGGAGA AGTATGGCAA ATGAAGGTTC TAATACTACT AGTGTAGGTG CAAATGCTCC AAATGCTGAT ACTATTGCTA GTGGAAGTCA
AAGTAGTACA AATAGTGCAA GTACTAGTAC TACTAATAAT GGAGAATCAC AAACTACTAC TCCTACCGCT GCTGATACTA TTGCTAGTGG
AAGTCAAAGG AGTACAAATA GTGCAAGTAC TAGTACTACT AATAATGGAG AATCACAAAC TACTACTCCT ACCGCTGCTG ATACTATTGC
TAGTGGAAGT CAAAGGAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC ACAAACTACT ACTCCTACCG CTGCTGATAC
CCCTACTGCT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG AAAGTTCAAG TTCTGGCAAT GCACCAAATA AAACAGACGG
TAAAGGAGAA GAGAGTGAAA AACAAAATGA ATTAAATGAA TCAACTGAAG AAGGACCCAA AGCTCCACAA GAACCTCAAA CGGCAGAAAA
TGAAAATCCT GCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA
TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA
TAAATTTGTT GTTTTAATTT CA

Figure 2: MRA-1255 K13 Sequence

ATCTGGTGGT AACAGCAATA GTGATGATAA AAGCGGAAGT AGTAGCGAGA ATGATTCTAA TTCATTTATG AATCTAACTA GTGATAAAAA
TGAGAAAACG GAAAATAATA GTTTCCTTTT AAATAATAGT AGTTATGGAA ATGTTAAAGA TAGCCTATTA GAATCCATTG ATATGAGTGT
ATTAGATTCG AACTTTGATA GTAAAAAAGA TTTTTTACCA AGTAATTTAT CAAGAACATT TAATAATATG TCTAAAGATA ATATAGGAAA
TAAATATTTA AATAAATTGT TAAATAAAAA AAAAGATACT ATTACAAATG AAAATAATAA TATTAATCAT AATAATAATA ATAATAATAA
TAATCTGACA GCAAATAATA TAACTAATAA TCTTATTAAT AATAATATGA ATTCTCCATC AATTATGAAT ACCAACAAAA AAGAGAATTT
TTTAGATGCA GCAAATCTTA TAAATGATGA TTCTGGATTA AACAATTTAA AAAAATTTTC AACTGTAAAT AATGTAAATG ATACTTATGA
AAAGAAAATT ATTGAAACGG AATTAAGTGA TGCTAGTGAT TTTGAAAATA TGGTAGGTGA TTTAAGAATT ACATTTATTA ATTGGTTAAA
AAAGACACAA ATGAATTTTA TTCGAGAAAA AGATAAATTA TTTAAAGATA AGAAAGAACT AGAAATGGAA AGAGTACGAT TGTACAAAGA
ATTAGAAAAC CGTAAAAATA TTGAAGAACA GAAATTACAT GATGAAAGAA AGAAATTAGA TATTGATATA TCTAATGGTT ATAAACAAAT
AAAAAAAGAA AAAGAAGAAC ATAGGAAACG ATTTGATGAA GAAAGATTAA GATTTTTACA AGAAATCGAT AAAATTAAAT TAGTATTATA
TTTAGAAAAA GAAAAATATT ATCAAGAATA TAAAAATTTT GAGAATGATA AAAAAAAAAT TGTTGATGCA AATATTGCTA CTGAAACTAT
GATTGATATT AATGTTGGTG GAGCTATTTT TGAAACATCT AGACATACCT TAACACAACA AAAAGATTCA TTTATAGAGA AATTATTAAG
TGGAAGACAT CATGTAACCA GAGATAAACA AGGAAGAATA TTCTTAGATA GGGATAGTGA GTTATTTAGA ATTATACTTA ACTTCTTAAG
AAATCCGTTA ACTATACCCA TACCAAAAGA TTTAAGTGAA AGTGAAGCCT TGTTGAAAGA AGCAGAATTT TATGGTATTA AATTTTTACC
ATTCCCATTA GTATTTTGTA TAGGTGGATT TGATGGTGTA GAATATTTAA ATTCGATGGA ATTATTAGAT ATTAGTCAAC AATGCTGGCG
TATGTGTACA CCTATGTCTA CCAAAAAAGC TTATTTTGGA AGTGCTGTAT TGAATAATTT CTTATACGTT TTTGGTGGTA ATAACTATGA
TTATAAGGCT TTATTTGAAA CAGAGGTGTA TGATAGATTA AGAGACGTCT GGTATGTATC AAGTAATTTA AATATACCTA GAAGAAATAA
TTGTGGTGTT ACGTCAAATG GTACAATTTA TTGTATTGGG GGATATGATG GCTCTTCTAT TATACCGAAT GTAGAAGCAT ATGATCATCG
TATGAAAGCA TGGGTAGAGG TGGCACCTTT GAATACCCCT AGATCATCAG CTATGTGTGT TGCTTTTGAT AATAAAATTT ATGTCATTGG
TGGAACTAAT GGTGAGAGAT TAAATTCTAT TGAAGTATAT GAAGAAAAAA TGAATAAATG GGAACAATTT CCATATGCCT TATTAGAAGC
TAGAAGTTCA GGAGCAGCTT TTAATTACCT TAATCAAATA TATGTTGTTG GAGGTATTGA TAATGAACAT AACATATTAG ATTCCGTTGA
ACAATATCAA CCATTTAATA AAAGATGGCA ATTTCTAAAT GGTGTACCAG AGAAAAAAAT GAATTTTGGA GCTGCCACAT TGTCAGATTC
TTATATAATT ACAGGAGGAG AAAATGGCGA AGTTCTAAAT TCATGTCATT TCTTTTCACC AGATACAAAT GAATGGCAGC TTGGCCCATC
TTTATTAGTT CCCAGATTTG GTCHCTCCG

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/Sonia Bjorum Brower/
Sonia Bjorum Brower 12 NOV 2025
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