Certificate of Analysis for MRA-1317

Plasmodium falciparum, Strain MRA1240-hap1

Catalog No. MRA-1317

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Product Description:

Plasmodium falciparum (P. falciparum), strain MRA1240-hap1 is a haplotype-specific drug response phenotype cloned from the multiclonal strain IPC 5202 (BEI Resources MRA-1240), which was originally isolated in 2011 from a human patient with malaria in Battambang Province, western Cambodia. MRA-1317 lot 70045870 was produced by cultivation of BEI Resources seed lot 70045871 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 grams per liter D-glucose, 27 µg per mL hypoxanthine and 5 µg per 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 13 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.

Lot: 70045870 Manufacturing Date: 29SEP2021

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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	40.6 ± 2.8 nM
Artemisinin	Report results	26.2 ± 0.6 nM
Quinine	Report results	216.2 ± 5.0 nM
Cycloguanil	Report results	788 ± 18.1 nM
Pyrimethamine	Report results	33980 ± 782.5 nM
Sulfadoxine	Report results	568500 ± 26190 nM
Ring-stage Survival Assay (RSA_{0-3h})[3]		
Dihydroartemisinin (DHA)	Report results	31.01%
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Sequencing of Kelch 13 (K13) gene (~ 2090 base pairs)	Contains C580Y or R539T mutation	Contains R539T mutation (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (13 days post-infection)[4]		
Ring-stage parasitemia	Report results	2.58%
Total parasitemia	≥ 2%	4.87%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	3.33%
Total parasitemia	≥ 1%	4.17%
Viability (3 days post-infection)[1]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[1]		
Harpo's HTYE broth, 37°C and 26°C, aerobic[5]	No growth	No growth
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
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth

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TEST	SPECIFICATIONS	RESULTS
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

[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. Available at: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx.]

[3]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.

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

[5]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1317 MSP2 Sequence

TTGTTACCTT TAATATTTAA 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 AGCTGAAAAW TCTGCTCCAA CAGCCGAACA
AACTGAATCC CCCGAATTAC AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA
AAATACTTMT GATAGTCAAA AAGAATGTAC CGATGGTAAC AAAGAAAA

Figure 2: MRA-1317 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 TATTAATMAT AATAATAATA ATAATAATCT
GACAGCAAAT AATATAACTA ATAATCTTAT TAATAATAAT ATGAATTCTC CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA
TGCAGCAAAT CTTATAAATG ATGATTCTGG ATTAAACAAT TTAAAAAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATTGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AAGAATTAGA
AAACCGTAAA AATATTGAAG AACAGAAATT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTTATAAAC AAATAAAAAA
AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAGA TTAAGATTTT TACAAGAAAT CGATAAAATT AAATTAGTAT TATATTTAGA
AAAAGAAAAA TATTATCAAG AATATAAAAA TTTTGAGAAT GATAAAAAAA AAATTGTTGA TGCAAATATT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTTGAAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGGAAG
ACATCATGTA ACCAGAGATA AACAAGGAAG AATATTCTTA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT TAAGAAATCC
GTTAACTATA CCCATACCAA AAGATTTAAG TGAAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAAATTTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TTAAATTCGA TGGAATTATT AGATATTAGT CAACAATGCT GGCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTATTT GAAACTGAGG TGTATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA 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 TTTGGTCAC

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Sonia Bjorum Brower 27 JUL 2022
Lead Technical Writer or designee, ATCC Federal Solutions

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