

***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% N₂, 5% CO₂, 5% O₂) 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 Microscopy¹	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)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% ⁴
Genotypic Analysis¹		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (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) ⁵		
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 ⁶	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

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 Contamination¹ DNA detection by PCR	None detected	None detected

¹Testing completed on vial, post-freeze material

²A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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>.]

³A 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>.

⁴RSA result level may be lower than reported value if the assay is performed with a high O₂ gas mixture.

⁵Testing completed on bulk material prior to vialing and freezing

⁶Atlas, 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

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TTGTTACCTT TAATATTTAA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGA
AGAAAGTAAT CTTTCTACTG 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 ACTACCACA CTACTAATGA TGCAGAAGCA TCTACCAGTA CCTCTTCAGA AAATCCAAAT CATAATAATG CCGAAACAAA
TCCAAAAGGT AAAGGAGAAG TTCAAAAACC AAATCAAGCA AATAAAGAAA CTCAAAATAA CTCAAATGTT CAACAAGACT CTCAAACTAA
ATCAAATGTT CCACCCACTC AAGATGCAGA CACTAAAAGT CCTACTGCAC AACCTGAACA AGCTGAAAAG TCTGCTCCAA CAGCCGAACA
AACTGAATCC CCCGAATTAC AATCTGCACC AGAGAATAAA GGTACAGGAC AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA
AAATACTTMT GATAGTCAA AAGAATGTAC CGATGGTAAC AAAGAAAA
    
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Figure 2: MRA-1317 K13 Sequence

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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 ATGAATTCCT CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA
TGCAGCAAAT CTTATAAATG ATGATTCCTGG ATTAACAAT TTAAAAAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AAGAATTAGA
AAACCGTAAA AATATTGAAG AACAGAAATT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAACC AAAATAAAAA
AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAAGA TTAAGATTTT TACAAGAAAT CGATAAAATT AAATTAGTAT TATATTTAGA
AAAAGAAAAA TATTATCAAG AATATAAAAA TTTTGAGAAT GATAAAAAAA AAATTGTTGA TGCAAAATATT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTGAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGAAG
ACATCATGTA ACCAGAGATA AACAAGGAAG AATATCTTGA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT TAAGAAATCC
GTAACTATA CCCATACCAA AAGATTTAAG TGAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAATTTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TTAATTCGA TGGAATTTAT AGATATTAGT CAACAATGCT GCGTATGTG
TACACCTATG TCTACCAAA AAGCTTATTT TGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTATTT GAAACTGAGG TGATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA TTTAAATATA CCTAGAAGAA ATAATTGTGG
TGTTACGTC AATGGTACA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT GTGTTGCTTT TGATAATAAA ATTTATGTCA TTGGTGAAC
TAATGGTGAG AGATTAAATT CTATTGAAGT ATATGAAGAA AAAATGAATA AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG
TTCAGGAGCA GCTTTAATT ACCTAATCA AATATATGTT GTTGAGGTA TTGATAATGA ACATAACATA TTAGATCCG TTGAACAATA
TCAACCATTT AATAAAAAGT GGCAATTTCT AAATGGTGTA CCAGAGAAAA AAATGAATTT TGGAGCTGCC ACATTGTCAG ATCTTATAT
AATTACAGGA GGAGAAAATG GCGAAGTTCT AAATTCATGT CATTTCTTTT CACCAGATAC AAATGAATGG CAGCTTGGCC CATCTTTATT
AGTTCCAGAA TTTGGTCAAC
    
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