

Plasmodium falciparum, Strain MRA1240-hap2

Catalog No. MRA-1318

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain MRA1240-hap2 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-1318 lot 70045872 was produced by cultivation of BEI Resources seed lot 70045873 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 14 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: 70045872

Manufacturing Date: 07SEP2021

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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 | 47.1 ± 3.3 nM |
| Artemisinin | Report results | 15.4 ± 0.7 nM |
| Quinine | Report results | 206.1 ± 14.2 nM |
| Cycloguanil | Report results | 803 ± 37.0 nM |
| Pyrimethamine | Report results | 28890 ± 1331nM |
| Sulfadoxine | Report results | 309100 ± 64515 nM |
| Ring-stage Survival Assay (RSA _{0-3h}) ³ | | |
| Dihydroartemisinin (DHA) | Report results | 21.89% |
| Genotypic Analysis¹ | | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 790 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 (14 days post-infection) ⁴ | | |
| Ring-stage parasitemia | Report results | 9.57% |
| Total parasitemia | ≥ 2% | 14.41% |
| Post-freeze (4 days post-infection) ¹ | | |
| Ring-stage parasitemia | Report results | 1.14% |
| Total parasitemia | ≥ 1% | 1.42% |
| 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 |
| Sheep blood agar, 37°C, aerobic | No growth | No growth |

| TEST | SPECIFICATIONS | RESULTS |
|---|----------------|---------------|
| 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>.

⁴Testing completed on bulk material prior to vialing and freezing

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

Figure 1: MRA-1318 MSP2 Sequence

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TACTAGAGTT ACTTAAGAGG GATGTTGCTG CTCACAGTT TTCTTTGTTA CCATCGGTAC ATTCTTTTGG ACTATCAGAA GTATTTTGTG
GATGATATATT TCTAGAACCA TGCATATGTC CATGTTGTCC TGTACCTTTA TTCTCTGGTG CAGATTGTAA TTCGGGGGAT TCAGTTTGTGTT
CGGCTGTTGG AGCAGAATTT TCAGCTTGTT CAGGTTGTGC AGTAGGACTT TTAGTGTCTG CATCTTGAGT GGGTGGAAACA TTTGATTTAG
TTTGAGAGTC TTGTTGAACA TTTGAGTTAT TTTGAGTTTC TTTATTTGCT TGATTTGGTT TTTGAACTTC TCCTTACCT TTTGGATTTG
TTTCGGCATT ATTATGATTT GGATTTTCTG AAGAGGTA CTGTTAGTACT GGTAGATGCT TCTGCATCAT TAGTAGTTGT GGTAGTTGTG GTAGTAGCGG
GAGTACTTGG ACTTCTCTCA GCATCTGCAC CAGGATTAGC ACCATTACCA TCACCAGAAC CAGCACTACC ACTACCACCA GCACTACCAC
TACCACCAGC ACTACCACCA CCACCAGCAC TACCACTACC ACCAGCACTA CCACTACCAC CAGCACTACC ACTACCACCA GCACTACCAC
TACCACCAGC ACTACCACCA CCACCAGCAC TACCACTACC ACCAGCACTA CCACTACCAC CAGCACTACC ACTACCACCA GCACTACCAC
TACTTCTCCT TATACTCATA TTATAAGCAT TGTTTATGAA TGTGTTGCTA TATTTACTTT CATTTTTAAAT ATTAAGG
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Figure 2: MRA-1318 K13 Sequence

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ATCTGGTGGT AACAGCAATA GTGATGATAA AAGCGGAAGT AGTAGCGAGA ATGATTCTAA TTCATTTATG AATCTAATA GTGATAAAAA
TGAGAAAACG GAAAATAATA GTTTCCTTTT AAATAATAGT AGTTATGGAA ATGTTAAAGA TAGCCTATTA GAATCCATTG ATATGAGTGT
ATTAGATTCG AACTTTGATA GTAAAAAGA TTTTACCCTA AGTAATTTAT CAAGAACATT TAATAATATG TCTAAAGATA ATATAGGAAA
TAAATATTTA AATAAATTGT TAAATAAAAA AAAAGATACT ATTACAAATG AAAATAATA TATTAATMAT AATAATAATA ATAATAATCT
GACAGCAAAAT AATATAACTA ATAATCTTAT TAATAATAAT ATGAATTTCT CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTAGA
TGCAGCAAAT CTTATAAATG ATGATTCTGG ATTAACAAT TTAAAAAAT TTTCACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATTGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AAGAATTAGA
AAACCGTAAA AATATTGAAG AACAGAAATT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAAC AAATAAAAA
AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAGA TTAAGATTTT TACAAGAAAT CGATAAAAT AAATTAGTAT TATATTTAGA
AAAAGAAAAA TATTATCAAG AATATAAAAA TTTGAGAAT GATAAAAAA AAATTGTTGA TGCAAAATAT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTGAAC ATCTAGACAT ACCTTAACAC AAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGGAA
ACATCATGTA ACCAGAGATA AACAAAGGAA AATATTCTTA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT TAAGAAATCC
GTTAACTATA CCCATACCA AAGATTTAAG TGAAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAATTTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TAAATTCGA TGAATTTAT AGATATTAGT CAACAATGCT GCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTTATT GAACTGAGG TGATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA TTTAAATATA CCTAGAAGAA ATAATTGTGG
TGTACGTC AATGGTACA TTTATTGTAT TGGGGATAT GATGGCTCT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT GTGTTGCTTT TGATAATAAA ATTTATGTCA TTGGTGGAA
TAATGGTGAG AGATTAATTT CTATTGAAGT ATATGAAGAA AAAATGAATA AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG
TTCAGGAGCA GCTTTTAAAT ACCTAATCA AATATATGTT GTTGGAGGTA TTGATAATGA ACATAACATA TTAGATTCCG TTGAACAATA
TCAACCATTT AATAAAAGAT GGCAATTTCT AAATGGTGTA CCAGAGAAAA AAATGAATTT TGGAGCTGCC ACATGTGTCAG ATCTTATAT
AATTACAGGA GGAGAAAATG GCGAAGTTCT AAATTCATGT CATTCTTTT CACCAGATAC AAATGAATGG CAGCTGGCC CATCTTTATT
AGTTCCAGA TTTGGTCA
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