

Plasmodium falciparum, Strain MRA1236-hap1

Catalog No. MRA-1315

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain MRA1236-hap1 is a haplotype-specific drug response phenotype cloned from the multiclonal strain IPC 3445 (BEI Resources MRA-1236), which was originally isolated in 2010 from the blood of a human patient with malaria in Pailin Province, western Cambodia. MRA-1315 lot 70045866 was produced by cultivation of BEI Resources seed lot 70045867 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 16 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70045866

Manufacturing Date: 09SEP2021

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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	26.8 ± 0.6 nM
Artemisinin	Report results	11.5 ± 0.3 nM
Quinine	Report results	158.7 ± 7.3 nM
Cycloguanil	Report results	1374 ± 158.5 nM
Pyrimethamine	Report results	38100 ± 1755 nM
Sulfadoxine	Report results	581900 ± 40228 nM
Ring-stage Survival Assay (RSA _{0-3h}) ³		
Dihydroartemisinin (DHA)	Report results	11.85%
Genotypic Analysis¹		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 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 C580Y mutation (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (16 days post-infection) ⁴		
Ring-stage parasitemia	Report results	11.15%
Total parasitemia	≥ 2%	16.54%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	0.56%
Total parasitemia	≥ 1%	1.69%
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-1315 MSP2 Sequence

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ATATTA AAAA TGAAAGTAAA TATAGCAACA CATTCATAAA CAATGCTTAT AAAATGAGTA TAAGGAGAAG TATGGCAAAT GAAGTTCTA
ATACTACTAG TGTAGGTGCA AATGCTCCAA ATGCTGATAC TATTGCTAGT GGAAGTCAAA GTAGTACAAA TAGTGCAAGT ACTAGTACTA
CTAATAATGG AGAATCACAA ACTACTACTC CTACCGCTGC TGATACCCCT ACTGCTACAA AAAGTAATTC ACCTTCACCA CCCATCACTA
CTACAGAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTAAT TCACCTTCAC CACCCATCAC TACTACAGAA AGTTCAAGTT
CTGGCAATGC ACCAATAAAA ACAGACGGTA AAGGAGAAGA GAGTAAAAAA AAAAATGAAT TAAATGAATC AACTGAAGAA GGACCCAAAG
CTCCACAAGA ACCTCAAACG GCAGAAAATG AAAATCCTGC TGCACCAGAG AATAAAGGTA CAGGACAACA TGGACATATG CATGGTTCTA
GAAATAATCA TCCACAAAAT ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG AAAACTGTGG AGCAGCAACA TCCCTCTTAA
ATAACTCTAG TAATATTGCT TCAATAAATA AATTGTGTGT TTTAATTTCA GCAACACTTG TTTTATCTTT TGCCATATTT CATATAAA
    
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Figure 2: MRA-1315 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 TTTTTTACCA AGTAATTTAT CAAGAACATT TAATAATATG TCTAAAGATA ATATAGGAAA
TAAATATTTA AATAAATGT TAAATAAAAA AAAAGATACT ATTACAAATG AAAATAATAA TATTAATMAT AATAATAATA ATAATAATCT
GACAGCAAAAT AATATAACTA ATAATCTTAT TAATAATAAT ATGAATTCCT CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA
TGCAGCAAAAT CTTATAAATG ATGATTCTGG ATTAAACAAT TTAAAAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATTGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AGAATTAGA
AAACCGTAAA AATATGAAG AACAGAAAT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAAC AAATAAAAA
AGAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAGA TTAAGATTTT TACAAGAAAT CGATAAAAT AAATTAGTAT TATATTTAGA
AAAAGAAAAA TATTATCAAG AATAAAAAA TTTTGAGAAT GATAAAAAAA AAATTGTTGA TGCAAAATAT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTGAAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGAAG
ACATCATGTA ACCAGAGATA AACAAGGAAG AATATTCTTA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAECTTCT TAAGAAATCC
GTAACTATA CCCATACCAA AAGATTTAAG TGAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAATTTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TAAATTCGA TGGAATTAT AGATATTAGT CAACAATGCT GCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTATTT GAAACTGAGG TGATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA TTTAAATATA CCTAGAAGAA ATAATTGTGG
TGTTACGTCA AATGGTAGAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGTATGT ATGTTGCTTT TGATAATAAA ATTTATGTCA TTGGTGAAC
TAATGGTGAG AGATTAATTT 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
AGTTCCAGAA TTTGGTCAAC
    
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