

Plasmodium falciparum, Strain MRA1285-hap3

Catalog No. MRA-1321

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Product Description:

Plasmodium falciparum (*P. falciparum*), strain MRA1285-hap3 is a haplotype-specific drug response phenotype cloned from the multiclonal strain IPC 6403 (BEI Resources MRA-1285), which was originally isolated in 2011 from a human patient with malaria in Battambang Province, western Cambodia. MRA-1321 lot 70045878 was produced by cultivation of BEI Resources seed lot 70045879 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 12 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: 70045878

Manufacturing Date: 20OCT2021

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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	83.6 ± 1.9 nM
Artemisinin	Report results	18.6 ± 1.0 nM
Quinine	Report results	136.2 ± 6.3 nM
Cycloguanil	Report results	1557 ± 216 nM
Pyrimethamine	Report results	34370 ± 2376 nM
Sulfadoxine	Report results	566800 ± 26111 nM
Ring-stage Survival Assay (RSA _{0-3h}) ³ Dihydroartemisinin (DHA)	Report results	11.15%
Genotypic Analysis¹		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 700 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 (12 days post-infection) ⁴		
Ring-stage parasitemia	Report results	2.95%
Total parasitemia	≥ 2%	4.56%
Post-freeze (2 days post-infection) ¹		
Ring-stage parasitemia	Report results	5.53%
Total parasitemia	≥ 1%	5.94%
Viability (2 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-1321 MSP2 Sequence

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TATTA AAAAT GAAAGTAAAT ATAGCAACAC ATTCATAAAC AATGCTTATA ATATGAGTAT AAGGAGAAGT ATGGCAAATG AAGGTTCTAA
TACTACTAGT GTAGGTGCAA ATGCTCCAAA TGCTGATACT ATTGCTAGTG GAAGTCAAAG TAGTACAAAAT AGTGCAAAGTA CTAGTACTAC
TAATAATGGA GAATCACAAA CTACTACTCC TACCGCTGCT GATACCCCTA CTGCTACAAA AAGTAATTCA CCTTCACCAC CCATCACTAC
TACAGAAAAGT AATTCACCTT CACCACCCAT CACTACTACA GAAAGTAATT CACCTTCACC ACCCATCACT ACTACAGAAA GTTCAAGTTC
TGGCAATGCA CCAAAATAAAA CAGACGGTAA AGGAGAAGAG AGTAAAAAAA AAAATGAATT AAATGAATCA ACTGAAAGAG GACCCAAAGC
TCCACAAGAA CCTCAAACGG CAGAAAATGA AAATCCTGCT GCACCAGAGA ATAAAGGTAC AGGACAACAT GGACATATGC ATGGTTCTAG
AAATAATCAT CCACAAAATA CTCTGATAG TCAAAAAGAA TGTACCGATG GTAACAAAGA AAACGTGGGA GCAGCAACAT CCCTCTTAAA
TAACTCTAGT AATATTGCTT CAATAAATAA ATTTGTTGTT TTAATTTTTCAG CAACACTTGT TTTATCTTTT GCCATA
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Figure 2: MRA-1321 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 GTAAAAAGA TTTTTTACCA AGTAATTTAT CAAGAACATT TAATAATATG TCTAAAGATA ATATAGGAAA
TAAATATTTA AATAAATGT TAAATAAAAA AAAAGATACT ATTACAAATG AAAATAATAA TATTAATMAT AATAATAATA ATAATAATCT
GACAGCAAT AATATAACTA ATAATCTTAT TAATAATAAT ATGAATTCCT CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA
TGCAGCAAT CTTATAAATG ATGATTCTGG ATTAACAACAT TTAAAAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATTGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AGAATTAGA
AAACCGTAAA AATATTGAAG AACAGAAAT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAAC AAATAAAAA
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AAAAGAAAA TATTATCAAG AATATAAAAA TTTTGAGAAT GATAAAAAA AAATTGTTGA TGCAAAATAT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTGAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGAAG
ACATCATGTA ACCAGAGATA AACAGGAAG AATATTCTTA GATAGGGATA GTGAGTTTAT TAGAATTATA CTTAACTTCT TAAGAAATCC
GTAACTATA CCCATACCAA AAGATTTAAG TGAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAATTTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TFAAATTCGA TGGAATTAT AGATATTAGT CAACAATGCT GCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGGAAAGTCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTATTT GAAACTGAGG TGATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA TTTAAATATA CCTAGAAGAA ATAATTGTGG
TGTTACGTC AATGGTAGAA TTTATGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGTATGT ATGTTGCTTT TGATAATAA ATTTATGTCA TTGGTGAAC
TAATGGTGAG AGATTAATTT CTATTGAAGT ATATGAAGAA AAAATGAATA AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG
TTCAGGAGCA GCTTTTAATT ACCTAATCA AATATATGTT GTTGGAGGTA TTGATAATGA ACATAACATA TTAGATTCCG TTGAACAATA
TCAACCATT AATAAAAGAT GGCAATTTCT AAATGGTGTA CCAGAGAAAA AAATGAATTT TGGAGCTGCC ACATTGTCAG ATCTTATAT
AATTACAGGA GGAGAAAATG GCGAAGTTCT AAATTCATGT CATTTCTTTT CACCAGATAC AAATGAATGG CAGCTTGGCC CATCTTTATT
AGTTCCAGAA TTTGGTCAAC
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08 AUG 2022

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