Certificate of Analysis for MRA-1316

Plasmodium falciparum, Strain MRA1236-hap2

Catalog No. MRA-1316

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Product Description:

Plasmodium falciparum (P. falciparum), strain MRA1236-hap2 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-1316 lot 70045868 was produced by cultivation of BEI Resources seed lot 70045689 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, 4 grams per liter D-glucose, 0.005 µg per mL hypoxanthine and 2.5 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: 70045868 **Manufacturing Date: 20OCT2021**

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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	42.9 ± 1.0 nM
Artemisinin	Report results	18.6 ± 0.4 nM
Quinine	Report results	211.0 ± 9.7 nM
Cycloguanil	Report results	1375 ± 63.3 nM
Pyrimethamine	Report results	23090 ± 3738 nM
Sulfadoxine	Report results	360500 ± 24922 nM
Ring-stage Survival Assay (RSA_{0-3h})[3]		
Dihydroartemisinin (DHA)	Report results	25.25%
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 700 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 C580Y mutation (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (13 days post-infection)[4]		
Ring-stage parasitemia	Report results	2.94%
Total parasitemia	≥ 2%	4.57%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	4.27%
Total parasitemia	≥ 1%	4.67%
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

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TEST	SPECIFICATIONS	RESULTS
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-1316 MSP2 Sequence

TTAAAAATGA AAGTAAATAT AGCAACACAT TCATAAACAA TGCTTATAAT ATGAGTATAA GGAGAAGTAT GGCAAATGAA GGTTCTAATA
CTACTAGTGT AGGTGCAAAT GCTCCAAATG CTGATACTAT TGCTAGTGGA AGTCAAAGTA GTACAAATAG TGCAAGTACT AGTACTACTA
ATAATGGAGA ATCACAAACT ACTACTCCTA CCGCTGCTGA TACCCCTACT GCTACAAAAA GTAATTCACC TTCACCACCC ATCACTACTA
CAGAAAGTAA TTCACCTTCA CCACCCATCA CTACTACAGA AAGTAATTCA CCTTCACCAC CCATCACTAC TACAGAAAGT TCAAGTTCTG
GCAATGCACC AAATAAAACA GACGGTAAAG GAGAAGAGAG TAAAAAAAAA AATGAATTAA ATGAATCAAC TGAAGAAGGA CCCAAAGCTC
CACAAGAACC TCAAACGGCA GAAAATGAAA ATCCTGCTGC ACCAGAGAAT AAAGGTACAG GACAACATGG ACATATGCAT GGTTCTAGAA
ATAATCATCC ACAAAATACT TCTGATAGTC AAAAAGAATG TACCGATGGT AACAAAGAAA ACTGTGGAGC AGCAACATCC CTCTTAAATA
ACTCTAGTAA TATTGCTTCA ATAAATAAAT TTGTTGTTTT AATTTCAGCA ACACTTGTTT TATCTTTTGC CATA

Figure 2: MRA-1316 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 AATGGTAGAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT ATGTTGCTTT 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/
Sonia Bjorum Brower 25 JUL 2022
Lead Technical Writer or designee, ATCC Federal Solutions

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