# Certificate of Analysis for MRA-1319

## *Plasmodium falciparum*, Strain MRA1240-hap3

## Catalog No. MRA-1319

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## Product Description:

*Plasmodium falciparum (P. falciparum)*, strain MRA1240-hap3 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-1319 lot 70045874 was produced by cultivation of BEI Resources seed lot 70045875 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% $N_2$, 5% $CO_2$, 5% $O_2$) 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: 70045874 Manufacturing Date: 28SEP2021

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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 | 69.4 ± 3.2 nM |
| Artemisinin | Report results | 18.1 ± 0.4 nM |
| Quinine | Report results | 251.3 ± 11.6 nM |
| Cycloguanil | Report results | 652.5 ± 45.1 nM |
| Pyrimethamine | Report results | 30160 ± 695 nM |
| Sulfadoxine | Report results | 465700 ± 42953 nM |
| Ring-stage Survival Assay ($RSA_{0\text{-}3h}$)[3] | | |
| Dihydroartemisinin (DHA) | Report results | 20.65% |
| **Genotypic Analysis**[1] | | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 850 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 R539T mutation (Figure 2) |
| **Level of Parasitemia by Giemsa Stain Microscopy** | | |
| Pre-freeze (12 days post-infection)[4] | | |
| Ring-stage parasitemia | Report results | 3.45% |
| Total parasitemia | ≥ 2% | 5.75% |
| Post-freeze (4 days post-infection)[1] | | |
| Ring-stage parasitemia | Report results | 3.78% |
| Total parasitemia | ≥ 1% | 4.49% |
| **Viability (4 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 |

**BEI Resources**
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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| 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 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-1319 MSP2 Sequence

AAAACATTGT CTATTATAAA TTTCTTTATT TTTGTTACCT TTAATATTAA AAATGAAAGT AAATATAGCA ACACATTCAT AAACAATGCT
TATAATATGA GTATAAGGAG AAGTATGGAA GAAAGTAATC CTTCTACTGG TGCTGGTGGT AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT
GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT
GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTTCT GGTGATGGTA ATGGTGCTAA TCCTGGTGCA
GATGCTGAGA GAAGTCCAAG TACTCCCGCT ACTACCACAA CTACCACAAC TACTAATGAT GCAGAAGCAT CTACCAGTAC CTCTTCAGAA
AATCCAAATC ATAATAATGC CGAAACAAAT CCAAAAGGTA AAGGAGAAGT TCAAAAACCA AATCAAGCAA ATAAAGAAAC TCAAAATAAC
TCAAATGTTC AACAAGACTC TCAAACTAAA TCAAATGTTC CACCCACTCA AGATGCAGAC ACTAAAAGTC CTACTGCACA ACCTGAACAA
GCTGAAAATT CTGCTCCAAC AGCCGAACAA ACTGAATCCC CCGAATTACA ATCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT
ATGCATGGTT CTAGAAATAA TCATCCACAA AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGAAAACTG TGGAGCAGCA
ACATCCCTCT TAAGTAACTC TAGTAATATT GCTTCAA

## Figure 2: MRA-1319 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 AATGGTACAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT GTGTTGCTTT 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 TTTGGTCMC

**BEI Resources**
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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/Sonia Bjorum Brower/
Sonia Bjorum Brower 05 AUG 2022
Lead Technical Writer or designee, ATCC Federal Solutions

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**BEI Resources**
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898