

***Plasmodium falciparum*, Strain Cam3.I_rev**

Catalog No. MRA-1252

Product Description:

Plasmodium falciparum (*P. falciparum*), strain Cam3.I_rev is a K13-propeller revertant mutant of the original Cam3.I strain, featuring a reversion in wild-type allele R539T. The original Cam3.I strain (also referred to as IPC 5202), was isolated in 2011 from a human patient with malaria in Battambang province, western Cambodia. *P. falciparum*, strain Cam3.I_rev was deposited as susceptible to artemisinin. MRA-1252 lot 70066719 was produced by cultivation of the BEI Resources seed lot 63268021 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 g/L D-glucose, 27 µg/mL hypoxanthine and 5 µg/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 19 days. Every 2 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: 70066719

Manufacturing Date: 28MAY2024

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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 | 65 ± 3.0 nM |
| Artemisinin | Report results | 27.3 ± 1.9 nM |
| Quinine | Report results | 284 ± 13.1 nM |
| Cycloguanil | Report results | 1047 ± 48.2 nM |
| Pyrimethamine | Report results | 27420 ± 3164 nM |
| Sulfadoxine | Report results | 207500 ± 38439 nM |
| Genotypic Analysis¹ | | |
| Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 base pairs) | Consistent with <i>P. falciparum</i> | Consistent with <i>P. falciparum</i> (Figure 1) |
| Sequencing of kelch protein (K13-propeller) gene (~ 2100 base pairs) ³ | Reversion of R539T mutation | Reversion of R539T mutation (Figure 2) |
| Level of Parasitemia by Giemsa Stain Microscopy | | |
| Pre-freeze (19 days post-infection) ⁴ | | |
| Ring-stage parasitemia | Report results | 3.37% |
| Total parasitemia | ≥ 2% | 5.52% |
| Post-freeze (2 days post-infection) ¹ | | |
| Ring-stage parasitemia | Report results | 2.65% |
| Total parasitemia | ≥ 1% | 2.90% |
| Viability (2 days post-infection)¹ | Growth in infected red blood cells | Growth in infected red blood cells |
| Sterility (14-day incubation)¹ | | |
| 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 |
| 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 |

| TEST | SPECIFICATIONS | RESULTS |
|---|----------------|---------------|
| 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. *Methods in Malaria Research Sixth Edition* is available on the [BEI Resources website](http://www.beiresources.org).]

³K13-propeller mutation R539T confers artemisinin resistance in the parental strain (MRA-1240). Threonine (ACA) in MRA-1240 is substituted by arginine (AGA) at codon 539 of the K13 gene in MRA-1252, contributing to the reversion to the wild-type sequence. For additional information, please refer to Straimer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." *Science*. 347 (2015): 428-431. PubMed: 25502314.

⁴Testing completed on bulk material prior to vialing and freezing

Figure 1: MRA-1252 MSP2 Sequence

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ATAAACATT GTCTATTATA AATTTCTTTA TTTTGTGTAC CTTTAATATT AAAAAATGAA GTAAATATAG CAACACATTC ATAAACAATG
CTTATAATAT GAGTATAAGG AGAAGTATGG AAGAAAGTAA TCCTTCTACT GGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG
CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG
CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTT CTGGTGATGG TAATGGTGCT AATCCTGGTG
CAGATGCTGA GAGAAGTCCA AGTACTCCCG CTACTACCAC AACTACCACA ACTACTAATG ATGCAGAAGC ATCTACCAGT ACCTCTTCAG
AAAATCCAAA TCATAATAAT GCCGAAACAA ATCCAAAAGG TAAAGGAGAA GTTCAAAAAC CAAATCAAGC AAATAAAGAA ACTCAAAATA
ACTCAATGT TCAACAAGAC TCTCAAAC TAATCAAATGT TCCACCCACT CAAGATGCAG AACTTAAAAG TCCTACTGCA CAACCTGAAC
AAGCTGAAAA TTCTGCTCCA ACAGCCGAAC AAATGAATC CCCCGAATYA CAATCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC
ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATAC

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Figure 2: MRA-1252 K13 Sequence

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GGGAATCTGG TGGTACACAG AATAGTGATG ATAAAAGCGG AAGTAGTAGC GAGAATGATT CTAATTCATT TATGAATCTA ACTAGTGATA
AAAATGAGAA AACGGAAAAAT AATAGTTTCC TTTTAAATAA TAGTAGTTAT GGAAATGTTA AAGATAGCCT ATTAGAATCC ATTGATATGA
GTGTATTAGA TTCGAAC TTTT GATAGTAAAA AAGATTTTTT ACCAAGTAAT TTATCAAGAA CATTTAATAA TATGTCTAAA GATAATATAG
GAAATAAATA TTTAAATAA TTGTTAAATA AAAAAAAGA TACTATTACA AATGAAAAA ATAATATTA TCATAATAAT AATAATAATA
ATAATAATCT GACAGCAAAAT AATATAACTA TAATCTTTAT TAATAATAAT ATGAATTCCT CATCAATTAT GAATACCAAC AAAAAAGAGA
ATTTTTTAGA TGCAGCAAAAT CTTATAAATG ATGATTCTGG ATTAACAAT TTAATAAAT TTTCAACTGT AAATAATGTA AATGATACTT
ATGAAAAGAA AATTATTGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT
TAAAAAAGAC ACAAATGAAT TTTATTCGAG AAAAAAGATA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA
AAGAATTAGA AAACCGTAAA AATATTGAAG AACAGAAAT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAAC
AAATAAAAA AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAGA TTAAGATTTT TACAAGAAAT CGATAAAAT AAATTAGTAT
TATATTTAGA AAAAGAAAA TATTATCAAG AATATAAAAA TTTTGAGAAAT GATAAAAAA AAATTGTGTA TGCAAAATATT GCTACTGAAA
CTATGATTGA TATTAATGTT GGTGGAGCTA TTTTGAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT
TAAGTGGGAG ACATCATGTA ACCAGAGATA AACAGGAAG AATATTCTTA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT
TAAGAAATCC GTTAACTATA CCCATACCAA AAGATTTAAG TGAAGTGAA GCCTTGTGTA AAGAAGCAGA ATTTTATGGT ATTAATTTT
TACCATTCCC ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TTAATTCGA TGGAATTATT AGATATTAGT CAACAATGCT
GGCGTATGTG TACACCTATG TCTACCAAAA AAGCTTATTT TGGAAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT
ATGATTATAA GGCTTTATTT GAAACAGAGG TGTATGATAG ATTAAGAGAC GTCTGGTATG TATCAAGTAA TTTAAATATA CCTAGAAGAA
ATAAATTGTTG TGTTACGTCA AATGGT AGAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC
ATCGTATGAA AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT GTGTTGCTTT TGATAATAAA ATTTATGTCA
TTGGTGGAAC TAATGGTGAG AGATTTAAT 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 GGAGAAAAATG GCGAAGTTCT AAATTCATGT CATTCTTTT CACCAGATAC AAATGAATGG CAGCTGGCC
CATCTTTATT AGTTCCAGA TTTGGTCMCT CCG

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