

Plasmodium falciparum, Strain IPC_6261

Catalog No. MRA-1284

Product Description:

Plasmodium falciparum (*P. falciparum*), strain IPC_6261 was isolated in 2012 from the blood of a human patient with malaria in Cambodia.

Lot: 70003872¹

Manufacturing Date: 30MAY2018

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy^{2,3}	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>)² Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ⁴ Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine Piperaquine Survival Assay (PSA) survival rate ⁵	Report results Report results Report results Report results Report results Report results Report results	175.4 ± 40.7 nM 14.0 ± 2.3 nM 127.7 ± 23.7 nM 626.5 ± 101.4 nM 7009.0 ± 1298.4 nM 30390.0 ± 49195 nM 39.6%
Genotypic Analysis² Sequencing of Merozoite Surface Protein 2 (MSP2) gene (820 base pairs) Sequencing of kelch protein (K13-propeller) gene (~ 2090 base pairs) ⁶	Consistent with <i>P. falciparum</i> Contains K13 C580Y mutation	Consistent with <i>P. falciparum</i> (Figure 1) Contains K13 C580Y mutation (Figure 2)
Functional Activity by PCR Amplification² MSP2 PCR amplicon analysis ⁷	~ 600 to 900 base pair amplicon	~ 850 base pair amplicon
Level of Parasitemia Pre-freeze ^{8,9} Ring-stage parasitemia Total parasitemia Post-freeze ^{2,10} Ring-stage parasitemia Total parasitemia	Report results ≥ 2% Report results ≥ 1%	2.43% 4.12% 1.36% 2.14%
Viability^{2,11}	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)² Harpo's HTYE broth, 37°C and 26°C, aerobic ¹² Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination² DNA Detection by PCR	None detected	None detected

¹MRA-1284 was produced by cultivation of BEI Resources MR-MRA-1284 lot 70003869 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 g/L D-glucose, 0.005 µg/mL hypoxanthine and 2.5 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 daily for (6 days). Every 1 to 4 days, uninfected, leukocyte filtered, Type A+ erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

- ²Testing completed on viald post-freeze material.
- ³Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 4 days.
- ⁴A SYBR Green [®] 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 [®]-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>].
- ⁵*P. falciparum*, strain IPC_6261 was reported with a PSA survival rate of 70.6% [Duru, V., et al. "Plasmodium falciparum Dihydroartemisinin-Piperazine Failures in Cambodia Are Associated with Mutant K13 Parasites Presenting High Survival Rates in Novel Piperazine *In Vitro* Assays: Retrospective and Prospective Investigations." *BMC Med.* 13 (2015): 305. PubMed: 26695060.].
- ⁶K13-propeller mutation C580Y confers artemisinin resistance *in vitro*; for additional information, please refer to Stramer, J., et al. "Drug Resistance. K13-Propeller Mutations Confer Artemisinin Resistance in *Plasmodium falciparum* Clinical Isolates." *Science* 347 (2015): 428-431. PubMed: 25502314.
- ⁷Primer sequences and conditions for PCR are available upon request.
- ⁸Testing completed on bulk material prior to vialing and freezing.
- ⁹Parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
- ¹⁰Parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
- ¹¹Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
- ¹²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1284 MSP2 Sequence

```
CCTTTAATAT TAAAAAATGA AAGTAAATAT AGCAACACAT TCATAAACAA TGCTTATAAT ATGAGTATAA GGAGAAGTAT
GGAAGAAAGT AATCCTTCTA CTGGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG
CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG GTAGTGCTGG TGGTAGTGGT AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT
AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG GTAGTGCTGG TTCTGGTGAT GGTAATGGTG CTAATCCTGG
TGCAGATGCT GAGAGAAGTC CAAGTACTCC CGCTACTACC ACAACTACCA CAACTACTAA TGATGCAGAA GCATCTACCA
GTACCTCTTC AGAAAATCCA AATCATAATA ATGCCGAAAC AAATCCAAAA GGTAAAGGAG AAGTTCAAAA ACCAAATCAA
GCAAAATAAG AAAC TCAAAA TAACTCAAAT GTTCAACAAG ACTCTCAAAC TAAATCAAAT GTTCCACCCA CTCAAGATGC
AGACACTAAA AGTCCTACTG CACAACCTGA ACAAGCTGAA AATTCTGCTC CAACAGCCGA ACAAAGTCAA TCCCCCGAAT
TACAATCTGC ACCAGAGAAT AAAGGTACAG GACAACATGG ACATATGCAT GGTTCAGAAA ATAATCATCC ACAAATAACT
TCTGATAGTC AAAAAGAATG TACCGATGGT AACAAAGAAA ACTGTGGAGC AGCAACATCC CTCTTAAGTA ACTCTAGTAA
TATTGCTTCA ATAAATAAAT
```

Figure 2: MRA-1284 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 TATTAATCAT AATAATAATA ATAATAATCT GACAGCAAAAT AATATAACTA ATAATCTTAT TAATAATAAT
ATGAATTCTC CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA TGCAGCAAAAT CTTATAAATG ATGATTCTGG
ATTAACAAT TTAATAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA AATTATTGAA ACGGAATTAA
GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTGGT TAAAAAGAC ACAAATGAAT
TTTATTCTGAG AAAAAGATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AAGAATTAGA
AAACCCGAAA AATATTGAAG AACAGAAATT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTTATAAAC
AAATAAAAAA AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAGA TTAAGATTTT TACAAGAAAT GATAAAAAAT
AAATTAGTAT TATATTTAGA AAAAAGAAAA TATTATCAAG AATATAAAAA TTTTGAGAAAT GATAAAAAAA AAATTGTTGA
TGCAAAATAT GCTACTGAAA CTATGATTGA TATTAATGTT GGTGGAGCTA TTTTTGAAAC ATCTAGACAT ACCTTAACAC
AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGGAAAG ACATCATGTA ACCAGAGATA AACAAGGAAG AATATTCTTA
GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT TAAGAAATCC GTTAACTATA CCCATACCAA AAGATTTAAG
TGAAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTAATTTTT TACCATTCCC ATTAGTATTT TGTATAGGTG
GATTTGATGG TGTAGAATAT TTAATTCGA TGAATTTATT 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 TTGGTGAAC TAATGGTGAG AGATTAAATT CTATTGAAGT ATATGAAGAA AAAATGAATA
 AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG TTCAGGAGCA GCTTTTAATT ACCTTAATCA AATATATGTT
 GTTGGAGGTA TTGATAATGA ACATAACATA TTAGATTCCG TTGAACAATA TCAACCATTT AATAAAAAGAT GGCAATTTCT
 AAATGGTGTA CCAGAGAAAA AAATGAATTT TGGAGCTGCC ACATTGTCAG ATTCTTATAT AATTACAGGA GGAGAAAATG
 GCGAAGTTCT AAATTCATGT CATTCTTTT CACCAGATAC AAATGAATGG CAGCTTGGCC CATCTTTATT AGTTCCCAGA
 TTTGGTCACT CC

/Heather Couch/

Heather Couch

20 JAN 2020

Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

