

Plasmodium falciparum, Strain IPC_6403

Catalog No. MRA-1285

Product Description:

Plasmodium falciparum (*P. alciparum*), strain IPC_6403 was isolated in 2013 from the blood of a human patient with malaria in Cambodia. MRA-1285 lot 70003874 was produced by inoculation of deposited material 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 16 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: 70003874

Manufacturing Date: 01FEB2018

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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	42.4 ± 3.9 nM
Artemisinin	Report results	12.6 ± 0.9 nM
Quinine	Report results	61.6 ± 8.5 nM
Cycloguanil	Report results	552.6 ± 25.5 nM
Pyrimethamine	Report results	13380 ± 925 nM
Sulfadoxine	Report results	281700 ± 19475 nM
Piperazine Survival Assay (PSA) survival rate ³	Report results	1.82%
Genotypic Analysis¹		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 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) ⁴	Contains C580Y mutation	Contains C580Y mutation (Figure 2)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (16 days post-infection) ⁵		
Ring-stage parasitemia	Report results	2.82%
Total parasitemia	≥ 2%	3.95%
Post-freeze (4 days post-infection) ¹		
Ring-stage parasitemia	Report results	2.47%
Total parasitemia	≥ 1%	3.01%
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 ⁶	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
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).]

³*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.].

⁴K13-propeller mutation C580Y confers artemisinin resistance *in vitro*; 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.

⁵Testing completed on bulk material prior to vialing and freezing

⁶Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1285 MSP2 Sequence

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AAATGAAAGT AAATATAGCA ACACATTCAT AAACAATGCT TATAATATGA GTATAAGGAG AAGTATGGCA AATGAAGGTT CTAATACTAC
TAGTGTAGGT GCAAATGCTC CAAATGCTGA TACTATTGCT AGTGGAAGTC AAAGTAGTAC AAATAGTGCA AGTACTAGTA CTACTAATAA
TGGAGAATCA CAAACTACTA CTCTTACCGC TGCTGATACC CCTACTGCTA CAAAAGTAA TTCACCTTCA CCACCCATCA CTACTACAGA
AAGTAATTCA CCTTACCAC CCATCACTAC TACAGAAAGT AATTCACCTT CACCACCCAT CACTACTACA GAAAGTTCAA GTTCTGGCAA
TGCACCAAAT AAAACAGACG GTAAAGGAGA AGAGAGTAAA AAAAAAATG AATTAATGA ATCAACTGAA GAAGGACCCA AAGCTCCACA
AGAACCCTCA ACGGCAGAAA ATGAAAATCC TGCTGCACCA GAGAATAAAG GTACAGGACA ACATGGACAT ATGCATGGTT CTAGAAATAA
TCATCCACAA AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA AAGAAAACGT TGGAGCAGCA ACATCCCTCT TAAATAACTC
TAGTAATATT GCTTCAATAA ATAAATTTGT TGTTTTAATT TCAGCAACAC TTGTTTTTATC TTTT
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Figure 2: MRA-1285 K13 Sequence

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ATCTGGTGGT AACAGCAATA GTGATGATAA AAGCGGAAGT AGTAGCGAGA ATGATTCTAA TTCATTTATG AATCTAACTA GTGATAAAAA
TGAGAAAACG GAAAAATAA GTTTCCTTTT AAATAATAGT AGTTATGGAA ATGTTAAAGA TAGCCTATTA GAATCCATTG ATATGAGTGT
ATTAGATTCG AACTTTGATA GTAAAAAGA TTTTTTACCA AGTAATTTAT CAAGAACATT TAATAATATG TCTAAAGATA ATATAGGAAA
TAAATATTTA AATAAATTGT TAAATAAAAA AAAAGATACT ATTACAAATG AAAATAATAA TATTAATCAT AATAATAATA ATAATAATCT
GACAGCAAAT AATATAACTA ATAATCTTAT TAATAATAAT ATGAATTTCT CATCAATTAT GAATACCAAC AAAAAAGAGA ATTTTTTAGA
TGCAGCAAAT CTTATAAATG ATGATTCTGG ATTAAACAAT TTAATAAAT TTTCAACTGT AAATAATGTA AATGATACTT ATGAAAAGAA
AATTATGAA ACGGAATTAA GTGATGCTAG TGATTTTGAA AATATGGTAG GTGATTTAAG AATTACATTT ATTAATTTGG TAAAAAGAC
ACAAATGAAT TTTATTCGAG AAAAAATAA ATTATTTAAA GATAAGAAAG AACTAGAAAT GGAAAGAGTA CGATTGTACA AAGAATTAGA
AAACCGTAAA AATATTGAAG AACAGAAATT ACATGATGAA AGAAAGAAAT TAGATATTGA TATATCTAAT GGTATAAAAC AAATAAAAAA
AGAAAAAGAA GAACATAGGA AACGATTTGA TGAAGAAAGA TTAAGATTTT TACAAGAAAT CGATAAAAT AAATTAGTAT TATATTTAGA
AAAAGAAAAA TATTATCAAG AATATAAAAA TTTTGAGAAT GATAAAAAAA AAATTGTTGA TGCAAAATAT GCTACTGAAA CTATGATTGA
TATTAATGTT GGTGGAGCTA TTTTGGAAAC ATCTAGACAT ACCTTAACAC AACAAAAAGA TTCATTTATA GAGAAATTAT TAAGTGGAAAG
ACATCATGTA ACCAGAGATA AACAAAGGAA AATATTCTTA GATAGGGATA GTGAGTTATT TAGAATTATA CTTAACTTCT TAAGAAATCC
GTTAAGTATA CCCATACCAA AAGATTTAAG TGAAGTGAA GCCTTGTTGA AAGAAGCAGA ATTTTATGGT ATTTAAATTT TACCATTCCC
ATTAGTATTT TGTATAGGTG GATTTGATGG TGTAGAATAT TTAATTTCTGA TGGAATTTAT AGATATTAGT CAACAATGCT GCGTATGTG
TACACCTATG TCTACCAAAA AAGCTTATTT TGAAGTGCT GTATTGAATA ATTTCTTATA CGTTTTTGGT GGTAATAACT ATGATTATAA
GGCTTTATTT GAACTGAGG TGATGATCG TTTAAGAGAT GTATGGTATG TTTCAAGTAA TTTAAATATA CCTAGAAGAA ATAATTGTGG
TGTTACGTC AATGGTAGAA TTTATTGTAT TGGGGGATAT GATGGCTCTT CTATTATACC GAATGTAGAA GCATATGATC ATCGTATGAA
AGCATGGGTA GAGGTGGCAC CTTTGAATAC CCCTAGATCA TCAGCTATGT ATGTTGCTTT TGATAATAAA ATTTATGTCA TTGGTGGAAC
TAATGGTGAG AGATTAATTT CTATTGAAGT ATATGAAGAA AAAATGAATA AATGGGAACA ATTTCCATAT GCCTTATTAG AAGCTAGAAG
TTCAGGAGCA CTTTTAATT ACCTTAATCA AATATATGTT GTTGGAGGTA TTGATAATGA ACATAACATA TTAGATTCCG TTGAACAATA
TCAACCATTT AATAAAAGAT GGC AATTTCT AAATGGTGTA CCAGAGAAA AAATGAATTT TGGAGCTGCC ACATGTCTAG ATTCTTATAT
AATTACAGGA GGAGAAAATG GCGAAGTTCT AAATTCATGT CATTCTTTT CACCAGATAC AAATGAATGG CAGCTTGGCC CATCTTTATT
AGTTCCAGAA TTTGGTAC
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