b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-285

Plasmodium falciparum, Strain D6

Catalog No. MRA-285

This reagent is the tangible property of the U.S. Government.

Product Description:

Plasmodium falciparum (P. falciparum), strain D6 was collected in Sierra Leone and is generally considered drug sensitive with minor mefloquine resistance. MRA-285 was produced by cultivation of BEI Resources MR-MRA-285 lot 59155175 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 14 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: 62669861 **Manufacturing Date: 12JUN2014**

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	5.7 ± 0.1 nM
Artemisinin	Report results	14.9 ± 0.7 nM
Quinine	Report results	3.7 ± 0.3 nM
Cycloguanil	Report results	5.3 ± 0.4 nM
Pyrimethamine	Report results	30.9 ± 2.9 nM
Sulfadoxine	Report results	321500 ± 37096 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 680 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain D6 (GenBank: ABGY01002422.1)	99.9% sequence identity to *P. falciparum*, strain D6 (GenBank: ABGY01002422.1) (Figure 1)
MSP2 PCR amplicon analysis[1]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (14 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.73%
Total parasitemia	≥ 2%	4.61%
Post-freeze (3 days post-infection)[1]		
Ring-stage parasitemia	Report results	1.92%
Total parasitemia	≥ 1%	6.73%
Viability (post-freeze; 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[4]	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
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

BEI Resources
www.beiresources.org

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

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]Testing completed on bulk material prior to vialing and freezing

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

Figure 1: MRA-285 MSP2 Sequence

```
TGTTACCTTT AATATTAAAA ATGAAAGTAA ATATAGCAAC ACATTCATAA ACAATGCTTA TAATATGAGT ATAAGGAGAA
GTATGGCAAA TGAAGGTTCT AGTACTAATA GTGTAGGTGC AAATGCTCCA AAAGCTGATA CTATTGCTAG TGGAAGTCAA
AGTAGTACAA ATAGTGCAAG TACTAGTACT ACTAATAATA GAGAATCACA AACTACTACT CCTACCACTG CTGATACCCC
TACTGCTACA GAAAGTAATT CACCTTCACC ACCCATCGCT ACTACAGAAA GTAATTCACC TTCACCACCC ATCACTACTA
CAGAAAGTAA TTCACCTTCA CCACCCATCA CTACTACAGA AAGTTCAAGT TCTGGCAATG CACCAAATAA AACAGACGGT
AAAGGAGAAG AGAGTGAAAA ACAAAATGAA TTAAATGAAT CAACTGAAGA AGGACCCAAA GCTCCACAAG AACCTCAAAC
GGCAGAAAAT GAAAATCCTG CTGCACCAGA GAATAAAGGT ACAGGACAAC ATGGACATAT GCATGGTTCT AGAAATAATC
ATCCACAAAA TACTTCTGAT AGTCAAAAAG AATGTACCGA TGGTAACAAA GAAAACTGTG GAGCAGCAAC ATCCCTCTTA
AATAACTCTA GTAATATTGC TTCAATAAAT AAATTTG
```

/Heather Couch/
Heather Couch 15 JUL 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.