Certificate of Analysis for MRA-200

Plasmodium falciparum, Strain RO-33

Catalog No. MRA-200

Product Description: *Plasmodium falciparum (P. falciparum)*, strain RO-33 was isolated in 1987, cultured from a blood sample of a Swiss tourist who had visited Ghana (West Africa) and developed malaria symptoms nine days after his return.

Lot[1]: 70009209 **Manufacturing Date: 16OCT2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[3]		
Chloroquine	Report results	8.1 ± 0.4 nM
Artemisinin	Report results	8.2 ± 0.4 nM
Quinine	Report results	37.5 ± 3.5 nM
Cycloguanil	Report results	503.9 ± 34.8 nM
Pyrimethamine	Report results	24450 ± 1690.3 nM
Sulfadoxine	Report results	136900 ± 18974 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain RO-33 (GenBank: ABGU00006677)	100% sequence identity to *P. falciparum*, strain RO-33 (GenBank: ABGU00006677) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]		
Ring-stage parasitemia	Report results	2.61%
Total parasitemia	≥ 2%	5.02%
Post-freeze[6]		
Ring-stage parasitemia	Report results	0.00%
Total parasitemia	≥ 1%	2.81%
Viability (post-freeze)[7]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)		
Harpo's HTYE broth[8], 37°C and 26°C, aerobic	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose 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		
DNA Detection by PCR	None detected	None detected

[1]MRA-200 was produced by cultivation of BEI Resources MRA-MR-200 lot 64457965 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 6 days. Every 1 to 2 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

[2]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 3 days.

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

© 2018 American Type Culture Collection (ATCC). All rights reserved.

[3]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].
[4]Primer sequences and conditions for PCR are available upon request.
[5]Pre-freeze parasitemia was determined after 6 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 days post infection.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-200 MSP2 Sequence

```
TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA
GAAGTATGGA AGAAAGTAAG CCTCCTACTG GTGCTAGTGG TAGTGCTGGT TCTGGTTCTG GTTCTGGTGC TGTTGCTAGT
GCTGGTAATG GTGCTAATCC TGGTGCAGAT GCTGAGAGAA GTCCAAGTAC TCCCGCTACT CCCGCTACTA CCACAACTAC
CACAACTACC ACAACTACTA ATGATGCAGA AGCATCTACC AGTACCTCTT CAGAAAATCC AAATCATAAT AAAGCCGAAA
CAAATCCAAA AGGTAAAGGA GAAGTTCAAA AACCAAATCA AGCAAATAAA GAAACTCAAA ATAACTCAAA TGTTCAACAA
GACTCTCAAA CTAAATCAAA TGTTCCACCC ACTCAAGATG CAGACACTAA AAGTCCTACT GCACAACCTG AACAAGCTGA
AAATTCTGCT CCAACAGCCG AACAAACTGA ATCCCCCGAA TTACAATCTG CACCAGAGAA TAAAGGTACA GGACAACATG
GACATATGCA TGGTTCTAGA AATAATCATC CACAAAATAC TTCTGATAGT CAAAAAGAAT GTACCGATGG TAACAAAGAA
AACTGTGGAG CAGCAACATC CCTCTTAAAT AACTCTAGTA ATATTGCTTC AATAAATAAA TTTGTT
```

Heather Couch
Digitally signed by Heather Couch
Date: 2018.05.07 14:45:00 -04'00'
07 MAY 2018
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.

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

© 2018 American Type Culture Collection (ATCC). All rights reserved.