Certificate of Analysis for MRA-1169

Plasmodium falciparum, Strain Tanzania (2000708)

Catalog No. MRA-1169

Product Description: *Plasmodium falciparum (P. falciparum)*, strain Tanzania (2000708) was isolated in September 2006 from the blood of a 3-year-old patient with mild malaria in Morogoro, Tanzania, and adapted to culture at Seattle Biomedical Research Institute, Seattle, Washington, USA.

Lot[1]: 61535357 **Manufacturing Date: 12MAR2013**

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	15.2 ± 0.7 nM
Artemisinin	Report results	8.2 ± 0.2 nM
Quinine	Report results	40.5 ± 2.8 nM
Cycloguanil	Report results	988.6 ± 68.3 nM
Pyrimethamine	Report results	12500 ± 1442.3 nM
Sulfadoxine	Report results	457400 ± 63393 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 690 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain Tanzania (2000708) (GenBank: AOPR01000217.1)	100% sequence identity to *P. falciparum*, strain Tanzania (2000708) (GenBank: AOPR01000217.1) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 850 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]		
Ring-stage parasitemia	Report results	3.23%
Total parasitemia	≥ 2%	4.58%
Post-freeze[6]		
Ring-stage parasitemia	Report results	0.82%
Total parasitemia	≥ 1%	1.09%
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-1169 was produced by cultivation of the 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, 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 19 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

BEI Resources
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[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 4 days.
[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 19 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 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-1169 MSP2 Sequence

ACAATGCTTA TAATATGAGT ATAAGGAGAA GTATGTCAGA AAGTAAGCCT CCTACTGGTG CTAGTGGTAG TGCTGGTTCT
GGTTCTGGTG CTGTTGCTAG TGCTGGTAAT GGTGCTAATC CTGGTGCAGA TGCTAAGAGA AGTCCAAGTA CTCCCGCTAC
TCCCGCTACT CCCGCTACTC CCGCTACTCC CGCTACTCCC GCTACTCCCG CTACTACCAC AACTACCACA ACTACTAATG
ATGCAGAAGC ATCTACCAGT ACCTCTTCAG AAAATTCAAA TCATAATAAT GCCGAAACAA ATCCAAAAGG TAAAGGAGAA
GTTCAAGAAC CAAATCAAGC AAATAAAGAA ACTCAAAATA ACTCAAATGT TCAACAAGAC TCTCAAACTA AATCAAATGT
TCCACCCACT CAAGATGCAG ACACTAAAAG TCCTACTGCA CAACCTGAAC AAGCTGAAAA TTCTGCTCCA ACAGCCGAAC
AAACTGAATC CCCCGAATTA CAATCTGCAC CAGAGAATAA AGGTACAGGA CAACATGGAC ATATGCATGG TTCTAGAAAT
AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA CAAAGAAAAC TGTGGAGCAG CAACATCCCT
CTTAAATAAC TCTAGTAATA TTGCTTCAAT AAATAAATTT GTTGTTTTAA TT

Date: 09 OCT 2017 **Signature:**

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