Certificate of Analysis for MRA-152

Plasmodium falciparum, Strain 7G8

Catalog No. MRA-152

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain 7G8 was cloned from the IMTM22 strain by limiting dilution. The original IMTM22 strain was isolated from a 12-year-old male near Manaus, Brazil in 1980. *P. falciparum*, strain 7G8 is a gametocyte producer, and was deposited as chloroquine-sensitive and pyrimethamine-resistant.

Lot[1]: 64022144 **Manufacturing Date: 21JAN2016**

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	30.1 ± 0.7 nM
Artemisinin	Report results	2.6 ± 0.1 nM
Quinine	Report results	75.2 ± 3.5 nM
Cycloguanil	Report results	517.2 ± 23.8 nM
Pyrimethamine	Report results	29160 ± 3365 nM
Sulfadoxine	Report results	366200 ± 50754 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain 7G8 (GenBank: ABGZ02000545)	100% sequence identity to *P. falciparum*, strain 7G8 (GenBank: ABGZ02000545) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]	Report results	3.17%
Post-freeze[6]	> 1%	4.97%
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-152 was produced by cultivation of MR-MRA-152 lot 58593809 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 8 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% 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 6 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

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

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 8 days post infection by microscopic counts of Giemsa-stained blood smears.

[6]Post-freeze parasitemia was determined after 6 days post infection by microscopic counts of Giemsa-stained blood smears.

[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 6 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-152 MSP2 Sequence

AAGGTAATTA AAACATTGTC TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA
CACATTCATA AACAATGCTT ATAATATGAG TATAAGGAGA AGTATGGCAG AAAGTAATCC TTCTACTGGT GCTGGTGGTA
GTGGTAGTGC TGGTGGTAGT GGTAGTGCTG GTGGTAGTGG TAGTGCTGGT GGTAGTGGTA GTGCTGGTGG TAGTGGTAGT
GCTGGTTCTG GTGATGGTAA TGGTGCTAAT CCTGGTGCAG ATGCTGAGAG AAGTCCAAGT ACTCCCGCTA CTACCACAAC
TACCACAACT ACTAATGATG CAGAAGCATC TACCAGTACC TCTTCAGAAA ATCCAAATCA TAATAATGCC GAAACAAATC
CAAAAGGTAA AGGAGAAGTT CAAAAACCAA ATCAAGCAAA TAAAGAAACT CAAAATAACT CAAATGTTCA ACAAGACTCT
CAAACTAAAT CAAATGTTCC ACCCACTCAA GATGCAGACA CTAAAAGTCC TACTGCACAA CCTGAACAAG CTGAAAATTC
TGCTCCAATA GCCGAACAAA CTGAATCCCC CGAATTACAA TCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA
TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT
GGAGCAGCAC CATCCCTCTT AAGTAACTCT AGTAATATTG CTTCAATAAA TAAATT

Date: 31 MAY 2016 **Signature:**

BEI Resources Authentication

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.