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SUPPORTING INFECTIOUS DISEASE RESEARCH

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.MRA-152 was produced by cultivation of seed material in fresh human erythrocytes suspended in RPMI 1640 medium, adjusted to contain 10% (volume per volume) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 grams per liter D-glucose, 0.005 micrograms per mL hypoxanthine and 2.5 micrograms per 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 for 10 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: 70051090 **Manufacturing Date: 25MAR2022**

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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	31.6 ± 0.7 nM
Artemisinin	Report results	10.5 ± 0.2 nM
Quinine	Report results	95.5 ± 4.4 nM
Cycloguanil	Report results	477.8 ± 33.0 nM
Pyrimethamine	Report results	32470 ± 748 nM
Sulfadoxine	Report results	443200 ± 30640 nM
Genotypic Analysis[1]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 760 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain 7G8 (GenBank: ABGZ02000544 and ABGZ02000545)	99.97% sequence identity to *P. falciparum*, strain 7G8 (GenBank: ABGZ02000544 and ABGZ02000545) (Figure 1)
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (10 days post-infection)[3]		
Ring-stage parasitemia	Report results	3.17%
Total parasitemia	≥ 2%	5.08%
Post-freeze (4 days post-infection)[1]		
Ring-stage parasitemia	Report results	3.58%
Total parasitemia	≥ 1%	11.58%
Viability (post-freeze; 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[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

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[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 (greater than 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.]
[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-152 MSP2 Sequence

ATTAAAACAT TGTCTATTAT AAATTTCTTT ATTTTTGTTT ACCTTTAATA TTAAAAATGA AAGTAAATAT AGCAACACAT
TCATAAACAA TGCTTATAAT ATGAGTATAA GGAGAAGTAT GGCAGAAAGT AATCCTTCTA CTGGTGCTGG TGGTAGTGGT
AGTGCTGGTG GTAGTGGTAG TGCTGGTGGT AGTGGTAGTG CTGGTGGTAG TGGTAGTGCT GGTGGTAGTG GTAGTGCTGG
TTCTGGTGAT GGTAATGGTG CTAATCCTGG TGCAGATGCT GAGAGAAGTC CAAGTACTCC CGCTACTACC ACAACTACCA
CAACTACTAA TGATGCAGAA GCATCTACCA GTACCTCTTC AGAAAATCCA AATCATAATA ATGCCGAAAC AAATCCAAAA
GGTAAAGGAG AAGTTCAAAA ACCAAATCAA GCAAATAAAG AAACTCAAAA TAACTCAAAT GTTCAACAAG ACTCTCAAAC
TAAATCAAAT GTTCCACCCA CTCAAGATGC AGACACTAAA AGTCCTACTG CACAACCTGA ACAAGCTGAA AATTCTGCTC
CAATAGCCGA ACAAAMTGAA TCCCCCGAAT TACAATCTGC ACCAGAGAAT AAAGGTACAG GACAACATGG ACATATGCAT
GGTTCTAGAA ATAATCATCC ACAAAATACT TCTGATAGTC AAAAAGAATG TACCGATGGT AACAAAGAAA ACTGTGGAGC
AGCACCATCC CTCTTAAGTA ACTCTAGTAA TATTGCTTCA ATAAATAAAT T

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Lead Technical Writer or designee, ATCC Federal Solutions

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