

Certificate of Analysis for MRA-164

Plasmodium falciparum, Strain GC03

Catalog No. MRA-164

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

Product Description: *Plasmodium falciparum (P. falciparum)*, strain GC03 is a genetic cross progeny of *P. falciparum* strains HB3 and Dd2.

Lot¹: 62941857 Manufacturing Date: 18SEP2014

TEST	SPECIFICATIONS	RESULTS		
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Identification by Giemsa Stain Microscopy ^{2,3}	Blood-stage parasites present	Blood-stage parasites present		
Genotypic Analysis ² Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 640 base pairs)	Consistent with <i>P. falciparum</i>	Consistent with <i>P. falciparum</i> (Figure 1)		
Functional Activity by PCR Amplification ² MSP2 PCR amplicon analysis ⁴	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon		
Level of Parasitemia Pre-freeze ^{5,6}	Depart receipts	0.040/		
Ring-stage parasitemia Total parasitemia Post-freeze ^{2,7}	Report results ≥ 2%	9.91% 13.5%		
Ring-stage parasitemia Total parasitemia	Report results ≥ 1%	1.81% 2.89%		
Viability (post-freeze) ^{2,8}	Growth in infected red blood cells	Growth in infected red blood cells		
Sterility (21-day incubation) ² Harpo's HTYE broth ⁹ , 37°C and 26°C, aerobic Tryptic Soy broth, 37°C and 26°C, aerobic Sabouraud Dextrose broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep Blood agar, 37°C, aerobic Sheep Blood agar, 37°C, anaerobic	No growth	No growth		
Thioglycollate broth, 37°C, anaerobic	No growth	No growth		

¹MRA-164 was produced by cultivation of BEI Resources MR-164 lot 2256348 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia daily for 10 days. Every day, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

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²Testing completed on vialed post-freeze material.

³Blood-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.

⁴Primer sequences and conditions for PCR are available upon request.

⁵Testing completed on bulk material prior to vialing and freezing.

⁶Parasitemia was determined after 10 days post infection by microscopic counts of Giemsa-stained blood smears.

Parasitemia was determined after 3 days post infection by microscopic counts of Giemsa-stained blood smears.

⁸Viability was confirmed by examination of infected erythrocytes for parasitemia at 3 days post infection.

⁹Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.



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Figure 1: MRA-164 MSP2 Sequence

GAGTTATTTA	AGAGGGATGT	TGCTGCTCCA	CAGTTTTCTT	TGTTACCATC	GGTACATTCT	TTTTGACTAT	CAGAAGTATT
TTGTGGATGA	TTATTTCTAG	AACCATGCAT	ATGTCCATGT	TGTCCTGTAC	CTTTATTCTC	TGGTGCAGCA	GGATTTTCAT
TTTCTGCCGT	TTGAGGTTCT	TGTGGAGCTT	TGGGTCCTTC	TTCAGTTGAT	TCATTTAATT	CATTTTGTTT	TTCACTCTCT
TCTCCTTTAC	CGTCTGTTTT	ATTTGGTGCA	TTGCCAGAAC	TTGAACTTTC	TGTAGTAGTG	ATGGGTGGTG	AAGGTGAATT
ACTTTTTGTA	GTAGTGATGG	GTGGTGAAGG	TGAATTACTT	TCTGTAGTAG	TGATGGGTGG	TGAAGGTGAA	TTACTTTCTG
TAGCAGTAGG	GGTATCAGCA	GCGGTAGGAG	TAGTATTTTG	TGATTCTCCA	TTATTAGTAG	TACTAGTACT	TGCACTATTT
GTACTACTTT	GACTTCCACT	AGCAATAGTA	TCAGCTTTTG	GAGCATTTGC	ACCTACACTC	TTAGTATTAG	AACCTTCATT
TGCCATACTT	CTCCTTATAC	TCATATTATA	AGCATTGTTT	ATGAATGTGT	TGCTATATTT	ACTTTCATTT	TTAATATTAA
A							

/Heather Couch/

Heather Couch 22 JUN 2018

Program Manager or designee, ATCC Federal Solutions

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