

***Plasmodium falciparum*, Strain C188**

Catalog No. MRA-166

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain C188 is a genetic cross progeny of *P. falciparum* strains HB3 and Dd2; strain C188 is reported to be chloroquine-sensitive.

Lot¹: 63901358

Manufacturing Date: 30NOV2015

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (<i>in vitro</i>) Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR green I [®] drug sensitivity assay ³		
Chloroquine	Report results	8.7 ± 0.4 nM
Artemisinin	Report results	10.6 ± 0.5 nM
Quinine	Report results	99.8 ± 9.2 nM
Cycloguanil	Report results	1391 ± 160.5 nM
Pyrimethamine	Report results	28930 ± 3338 nM
Sulfadoxine	Report results	329500 ± 53339 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 840 base pairs) MSP2 PCR amplicon analysis ⁴	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon	Consistent with <i>P. falciparum</i> (Figure 1) ~ 840 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Post-freeze ⁶	Report results > 1%	4.40% 5.40%
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 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 Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹MRA-166 was produced by cultivation of MRA-166 lot 2287514 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 12 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.

²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.

³A SYBR Green I[®] anti-malarial drug sensitivity assay in 96-well plates was used to determine IC₅₀ 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.mr4.org/Publications/MethodsInMalariaResearch.aspx>].

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

⁵Pre-freeze parasitemia was determined after 12 days post infection by microscopic counts of Giemsa-stained blood smears.

⁶Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.

⁸Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-166 MSP2 Sequence

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TTTATTTTTG TTACCTTTAA TATTAAAAAT GAAAGTAAAT ATAGCAACAC ATTCATAAAC AATGCTTATA ATATGAGTAT
AAGGAGAAGT ATGGCAAATG AAGGTTCTAA TACTACTAGT GTAGGTGCAA ATGCTCCAAA TGCTGATACT ATTGCTAGTG
GAAGTCAAAG TAGTACAAAT AGTGCAAGTA CTAGTACTAC TAATAATGGA GAATCACAAA CTACTACTCC TACCGCTGCT
GATACTATTG CTAGTGGAAG TCAAAGGAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT CACAACTAC
TACTCCTACC GCTGCTGATA CTATTGCTAG TGGAAAGTCAA AGGAGTACAA ATAGTGCAAG TACTAGTACT ACTAATAATG
GAGAATCACA AACTACTACT CCTACCGCTG CTGATACCCC TACTGCTACA GAAAGTAATT CACCTTCACC ACCCATCACT
ACTACAGAAA GTTCAAGTTC TGGCAATGCA CCAAATAAAA CAGACGGTAA AGGAGAAGAG AGTGAAAAAC AAAATGAATT
AAATGAATCA ACTGAAGAAG GACCCAAAGC TCCACAAGAA CCTCAAACGG CAGAAAATGA AAATCCTGCT GCACCAGAGA
ATAAAGGTAC AGGACAACAT GGACATATGC ATGGTTCTAG AAATAATCAT CCACAAAATA CTTCTGATAG TCAAAAAGAA
TGTACCGATG GTAACAAAGA AAACGTGGA GCAGCAACAT CCCTCTTAAA TAACTCTAGT AATATTGCTT CAATAAATAA
ATTTGTTGTT TTAATTTTCAG CAACACTTGT TTTATCTTTT
    
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Date: 06 APR 2016

Signature: 

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