

Plasmodium falciparum, Strain ITG-2G2

Catalog No. MRA-326

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain ITG-2G2 was isolated in Brazil.

Lot¹: 63937216

Manufacturing Date: 22DEC2015

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 Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	45.0 ± 2.1 nM 13.1 ± 0.9 nM 339.4 ± 23.5 nM 876.7 ± 43.5 nM 24.4 ± 1.7 nM 514600 ± 83303 nM
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 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) ~ 800 base pair amplicon
Level of Parasitemia Pre-freeze ⁵ Post-freeze ⁶	Report results > 1%	3.44% 1.78%
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-326 was produced by cultivation of MRA-326 lot 2319626 in fresh human erythrocytes 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 5 days. Every 1 to 3 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.beiresources.org/Publications/MethodsInMalariaResearch.aspx>].

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

⁵Pre-freeze parasitemia was determined after 5 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-326 MSP2 Sequence

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ATTTATTTAT TGAAGCAATA TTACTAGAGT TATTTAAGAG GGATGTTGCT GCTCCACAGT TTTCTTTGTT ACCATCGGTA
CATTCTTTTT GACTATCAGA AGTATTTTGT GGATGATTAT TTCTAGAACC ATGCATATGT CCATGTTGTC CTGTACCTTT
ATTCTCTGGT GCAGATTGTA ATTCGGGGGA TTCAGTTTGT TCGGCTGTTG GAGCAGAATT TTCAGCTTGT TCAGGTTGTG
CAGTAGGACT TTTAGTGTCT GCATCTTGAG TGGGTGGAAC ATTTGATTTA GTTTGAGAGT CTTGTTGAAC ATTTGAGTTA
TTTTGAGTTT CTTTATTTGC TTGATTTGTT TCGGCATTAT TATGATTTGG ATTTTCTGAA GAGGTACTGG TAGATGCTTC
TGCATCATTA GTAGTTGTGG TAGTTGTGGT AGTAGCGGGA GTACTTGGAC TTCTCTCAGC ATCTGCACCA GGATTAGCAC
CATTACCATC ACCAGAACCA GCACTACCAC CAGCACTACC ACCAGCACTA CCACCAGCAC TACCACCAGC ACTACCACCA
GCACTACCAC CAGCACTACC ACCAGCACTA CCACCAGCAC TACCACCAGC ACTACCACCA GCACTACCAC CAGCACTACC
ACCAGCACTA CCACTAGCAC CAGTAGGAGG ATTACTTTCT GTCATACTTC TCCTTATACT CATATTATAA GCATTGTTTA
TGAATGTGTT GCTATATTTA CTTTCATTTT TAATATTTAA GGTAACAAAA ATAAAGAAAT TTATAATAGA CAATGTTTTA
AT
    
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Date: 10 MAY 2016

Signature: 

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