b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-843

Plasmodium falciparum, Strain Dd2^{attB}

Catalog No. MRA-843

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain Dd2^{attB} was generated by integration of the acceptor *attB* site, recognized by the mycobacteriophage Bxb1 integrase during site-specific integration, into the nonessential glutaredoxin-like *cg6* gene located on chromosome 7. *P. falciparum*, strain Dd2 originated in 1980 in Indochina.

Lot[1]: 63937215 **Manufacturing Date: 17DEC2015**

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	63.5 ± 2.9 nM
Artemisinin	Report results	11.9 ± 0.8 nM
Quinine	Report results	251.9 ± 34.9 nM
Cycloguanil	Report results	582.8 ± 135.4 nM
Pyrimethamine	Report results	26740 ± 1848.6 nM
Sulfadoxine	Report results	336900 ± 3107.4 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (860 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum*[4] (Figure 1)
MSP2 PCR amplicon analysis[5]	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze[6]	Report results	3.87%
Post-freeze[7]	> 1%	1.51%
Viability (post-freeze)[8]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)		
Harpo's HTYE broth[9], 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-843 was produced by cultivation of MR-MRA-843 lot 7606700 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 7 days. Every 1 to 2 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 5 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 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].

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[4]100% sequence identity to GenBank: AASM01000018 (*P. falciparum*, strain Dd2)
[5]Primer sequences and conditions for PCR are available upon request.
[6]Pre-freeze parasitemia was determined after 7 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.
[9]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-843 MSP2 Sequence

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CATTGTCTAT TATAAATTTC 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 CACAAACTAC TACTCCTACC GCTGCTGATA CTATTGCTAG TGGAAGTCAA 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 AAACTGTGGA GCAGCAACAT CCCTCTTAAA TAACTCTAGT
AATATTGCTT CAATAAATAA ATTTGTTGTT TTAATTTCAG CAACACTTGT TTTATCTTTT
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Date: 10 MAY 2016 **Signature:**

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