Certificate of Analysis for MRA-1229

Plasmodium falciparum, Strain BC4

Catalog No. MRA-1229

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain BC4 is a subclone of HB3B, which is a clonal parasite of the HB3 strain after mosquito and chimpanzee passage. *P. falciparum*, strain HB3 was cloned from the Honduras I/CDC strain, originally isolated from a patient in Choluteca, Honduras, during an outbreak of urban malaria in January 1980. *P. falciparum* strains HB3B and HB3 are available as BEI Resources MRA-1227 and MRA-155, respectively.

Lot[1]: 63750691 **Manufacturing Date: 13OCT2015**

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	11.7 ± 0.8 nM
Artemisinin	Report results	6.0 ± 0.3 nM
Quinine	Report results	58.1 ± 2.7 nM
Cycloguanil	Report results	42.1 ± 1.0 nM
Pyrimethamine	Report results	1620.0 ± 112.0 nM
Sulfadoxine	Report results	376200 ± 43407 nM
Genotypic Analysis		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 base pairs)	≥ 99% sequence identity to *P. falciparum,* strain HB3 (GenBank: AANS01000284.1)	100% sequence identity to *P. falciparum,* strain HB3 (GenBank: AANS01000284.1) (Figure 1)
MSP2 PCR amplicon analysis[4]	~ 600-900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia		
Pre-freeze[5]	Report results	2.69%
Post-freeze[6]	> 1%	4.42%
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	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-1229 was produced by cultivation of the deposited material 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% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 14 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.

[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 4 days.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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[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].
[4]Primer sequences and conditions for PCR are available upon request.
[5]Pre-freeze parasitemia was determined after 14 days post infection by microscopic counts of Giemsa-stained blood smears.
[6]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[7]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1229 MSP2 Sequence

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TAAAACATTG TCTATTATAA ATTTCTTTAT TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA
TAAACAATGC TTATAATATG AGTATAAGGA GAAGTATGGC AAATGAAGGT TCTAATACTA AGAGTGTAGG TGCAAATGCT
CCAAAAGCTG ATACTATTGC TAGTGGAAGT CAAAGTAGTA CAAATAGTGC AAGTACTAGT ACTACTAATA ATGGAGAATC
ACAAAATACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAGTA ATTCACCTTC ACCACCCATC ACTACTACAG
AAAGTAATTC ACCTTCACCA CCCATCACTA CTACAAAAAG TAATTCACCT TCACCACCCA TCACTACTAC AGAAAGTTCA
AGTTCTGGCA ATGCACCAAA TAAAACAGAC GGTAAAGGAG AAGAGAGTGA AAAACAAAAT GAATTAAATG AATCAACTGA
AGAAGGACCC AAAGCTCCAC AAGAACCTCA AACGGCAGAA AATGAAAATC CTGCTGCACC AGAGAATAAA GGTACAGGAC
AACATGGACA TATGCATGGT TCTAGAAATA ATCATCCACA AAATACTTCT GATAGTCAAA AAGAATGTAC CGATGGTAAC
AAAGAAAACT GTGGAGCAGC AACATCCCTC TTAAATAACT CTAGTAATAT TGCTTCAATA AATAAATTTG T
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Date: 26 APR 2016 **Signature:**

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