

Certificate of Analysis for MRA-155

Plasmodium falciparum, Strain HB3

Catalog No. MRA-155

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

Product Description:

Plasmodium falciparum (*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. MRA-155 lot 70049772 was produced by cultivation of BEI Resources seed lot 58243283 in fresh human erythrocytes suspended in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 2 g/L D-glucose, 27 μg/mL hypoxanthine and 5 μg/mL gentamicin. The culture was incubated at 37° C in sealed flasks outgassed with a blood-gas atmosphere (90% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia for 10 days. Every 1 to 3 days, uninfected, leukocyte-filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70049772 Manufacturing Date: 27JAN2022

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TEST	SPECIFICATIONS	RESULTS			
Identification by Giemsa Stain Microscopy ¹	Blood-stage parasites present	Blood-stage parasites present			
Antimalarial Susceptibility Profile (in vitro) ¹ Half-maximal Inhibitory Concentration (IC ₅₀) by SYBR Green I [®] drug sensitivity assay ²					
Chloroquine	Report results	11.5 ± 0.8 nM 9.7 ± 0.7 nM			
Artemisinin	Report results				
Quinine	Report results	98.3 ± 6.8 nM			
Cycloguanil	Report results	62.1 ± 5.7 nM			
Pyrimethamine	Report results	16780 ± 154.8 nM			
Sulfadoxine	Report results	387900 ± 35777 nM			
Genotypic Analysis¹ Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs)	≥ 99% sequence identity to P. falciparum, strain HB3 (GenBank: AANS01000284.1)	99.9% sequence identity to P. falciparum, strain HB3 (GenBank: AANS01000284.1)			
Level of Parasitemia by Giemsa Stain Microscopy					
Pre-freeze (10 days post-infection) ³					
Ring-stage parasitemia	Report results	4.71%			
Total parasitemia	≥ 2%	6.91%			
Post-freeze (4 days post-infection) ¹					
Ring-stage parasitemia	Report results	2.72%			
Total parasitemia	≥ 1%	4.47%			
Viability (4 days post-infection) ¹	Growth in infected red blood cells	Growth in infected red blood cells			
Sterility (21-day incubation) ¹					
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴	No growth	No growth			
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth			
Sabouraud 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			

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

Fax: 703-365-2898



SUPPORTING INFECTIOUS DISEASE RESEARCH

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TEST	SPECIFICATIONS	RESULTS		
Mycoplasma Contamination ¹ DNA detection by PCR	None detected	None detected		

¹Testing completed on vialed, post-freeze material

Figure 1: MRA-155 MSP2 Sequence

CCTTTAATAT	TAAAAATGAA	AGTAAATATA	GCAACACATT	CATAAACAAT	GCTTATAAAA	TGAGTATAAG	GAGAAGTATG	GCAAATGAAG	
GTTCTAATAC	TAAGAGTGTA	GGTGCAAATG	CTCCAAAAGC	TGATACTATT	GCTAGTGGAA	GTCAAAGTAG	TACAAATAGT	GCAAGTACTA	
GTACTACTAA	TAATGGAGAA	TCACAAAATA	CTACTCCTAC	CGCTGCTGAT	ACCCCTACTG	CTACAGAAAG	TAATTCACCT	TCACCACCCA	
TCACTACTAC	AGAAAGTAAT	TCACCTTCAC	CACCCATCAC	TACTACAAAA	AGTAATTCAC	CTTCACCACC	CATCACTACT	ACAGAAAGTT	
CAAGTTCTGG	CAATGCACCA	AATAAAACAG	ACGGTAAAGG	AGAAGAGAGT	GAAAAACAAA	ATGAATTAAA	TGAATCAACT	GAAGAAGGAC	
CCAAAGCTCC	ACAAGAACCT	CAAACGGCAG	AAAATGAAAA	TCCTGCTGCA	CCAGAGAATA	AAGGTACAGG	ACAACATGGA	CATATGCATG	
GTTCTAGAAA	TAATCATCCA	CAAAATACTT	CTGATAGTCA	AAAAGAATGT	ACCGATGGTA	ACAAAGAAAA	CTGTGGAGCA	GCAACATCCC	
TCTTAAATAA	CTCTAGTAAT	ATTGCTTCAA	TAAATAAATT	TGTTGTTTTA	${\tt ATTTCAGCAA}$	CACTTGTTTT	ATCTTTTGCC	ATATTCATAT	
AAA									

/Sonia Bjorum Brower/ Sonia Bjorum Brower

29 SEP 2022

Technical Manager or designee, ATCC Federal Solutions

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E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

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²A 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. Methods in Malaria Research Sixth Edition is available on the BEI Resources website.]

³Testing completed on bulk material prior to vialing and freezing

⁴Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.