Certificate of Analysis for MRA-568

Plasmodium falciparum, Strain D10 ACP_{leader}-GFP

Catalog No. MRA-568

Product Description:

Plasmodium falciparum (P. falciparum), strain D10 ACP_{leader}-GFP is a *P. falciparum*, strain D10 derivative that was created by transfection of the parent strain with a plasmid containing a fusion of green fluorescent protein (GFP) with the *P. falciparum* acyl carrier protein (ACP) leader peptide (using amino acids 1 through 60). *P. falciparum*, strain D10 ACP_{leader}-GFP was deposited as displaying cytoplasmic GFP fluorescence in merozoites through schizonts, and can be utilized as a tool to study protein trafficking and plastid targeting. MRA-568 was produced by cultivation of BEI Resources MR-MRA-568 lot 59005983 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 8 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: 70032351 **Manufacturing Date: 12FEB2020**

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	16.9 ± 3.1 nM
Artemisinin	Report results	5.4 ± 0.4 nM
Quinine	Report results	30.0 ± 2.8 nM
Cycloguanil	Report results	353.3 ± 65.4 nM
Pyrimethamine	Report results	49610 ± 4576 nM
Sulfadoxine	Report results	438300 ± 40426 nM
Genotypic Analysis[3]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 780 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Phenotypic Analysis		
GFP expression	Positive	Positive
Functional Activity by PCR Amplification[3]		
MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia		
Pre-freeze (8 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.68%
Total parasitemia	≥ 2%	3.89%
Post-freeze (2 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.90%
Total parasitemia	≥ 1%	1.20%
Viability (post-freeze; 2 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[2]	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

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Certificate of Analysis for MRA-568

TEST	SPECIFICATIONS	RESULTS
Mycoplasma Contamination[1] DNA Detection by PCR	None detected	None detected

[1]Testing completed on vialed, post-freeze material
[2]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: to https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx.
[3]Testing completed on bulk material prior to vialing and freezing
[4]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-568 MSP2 Sequence

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AATTAAAAAC ATTGTCTATT ATAAATTTCT TTATTTTTGT TACCTTTAAT ATTAAAAATG AAAGTAAATA TAGCAACACA
TTCATAAACA ATGCTTATAA TATGAGTATA AGGAGAAGTA TGGCAAATGA AGGTTCTAAT ACTAATAGTG TAGGTGCAAA
TGCTCCAAAT GCTGATACTA TTGCTAGTGG AAGTCAAAGG AGTACAAATA GTGCAAGTAC TAGTACTACT AATAATGGAG
AATCACAAAC TACTACTCCT ACCGCTGCTG ATACTATTGC TAGTGGAAGT CAAAGGAGTA CAAATAGTGC AAGTACTAGT
ACTACTAATA ATGGAGAATC ACAAACTACT ACTCCTACCG CTGCTGATAC CCCTACTGCT ACAGAAAGTA ATTCACCTTC
ACCACCCATC ACTACTACAG AAAGTTCAAG TTCTGGCAAT GCACCAAATA AAACAGACGG TAAAGGAGAA GAGAGTGAAA
AACAAAATGA ATTAAATGAA TCAACTGAAG AAGGACCCAA AGCTCCACAA GAACCTCAAA CGGCAGAAAA TGAAAATCCT
GCTGCACCAG AGAATAAAGG TACAGGACAA CATGGACATA TGCATGGTTC TAGAAATAAT CATCCACAAA ATACTTCTGA
TAGTCAAAAA GAATGTACCG ATGGTAACAA AGAAAACTGT GGAGCAGCAA CATCCCTCTT AAGTAACTCT AGTAATATTG
CTTCAATAAA TAAATTTGTT GTTTTAATTT CAGCAACACT TGTTTTATCT TTTGC
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/Heather Couch/
Heather Couch 25 MAY 2020
Program Manager or designee, ATCC Federal Solutions

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