Certificate of Analysis for MRA-569

Plasmodium falciparum, Strain D10 $ACP_{transit}$-GFP

Catalog No. MRA-569

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain D10 $ACP_{transit}$-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) pre-sequence minus the signal peptide domain (using amino acids 14 through 60). *P. falciparum*, strain D10 $ACP_{transit}$-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.

Lot[1]: 62941856 **Manufacturing Date: 25SEP2014**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis[2] Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 740 base pairs)	Consistent with *P. falciparum*	Consistent with *P. falciparum* (Figure 1)
Phenotypic Analysis GFP expression	Positive	Positive (Figure 2)
Functional Activity by PCR Amplification[2] MSP2 PCR amplicon analysis[4]	~ 600 to 900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia Pre-freeze[5,6] Ring-stage parasitemia Total parasitemia Post-freeze[2,7] Ring-stage parasitemia Total parasitemia	 Report results ≥ 2% Report results ≥ 1%	 2.66% 4.70% 2.71% 4.17%
Viability[2,8]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2] Harpo's HTYE broth[9], 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

[1]MRA-569 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 for 1 day. Every day, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.
[2]Testing completed on vialed post-freeze material
[3]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.
[4]Primer sequences and conditions for PCR are available upon request.
[5]Testing completed on bulk material prior to vialing and freezing
[6]Parasitemia was determined after 1 day post infection by microscopic counts of Giemsa-stained blood smears.
[7]Parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[9]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Figure 1: MRA-569 MSP2 Sequence

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TGGCAAAAGA TAAAACAAGT GTTGCTGAAA TTAAAACAAC AAATTTATTT ATTGAAGCAA TATTACTAGA GTTACTTAAG
AGGGATGTTG CTGCTCCACA GTTTTCTTTG TTACCATCGG TACATTCTTT TTGACTATCA GAAGTATTTT GTGGATGATT
ATTTCTAGAA CCATGCATAT GTCCATGTTG TCCTGTACCT TTATTCTCTG GTGCAGCAGG ATTTTCATTT TCTGCCGTTT
GAGGTTCTTG TGGAGCTTTG GGTCCTTCTT CAGTTGATTC ATTTAATTCA TTTTGTTTTT CACTCTCTTC TCCTTTACCG
TCTGTTTTAT TTGGTGCATT GCCAGAACTT GAACTTTCTG TAGTAGTGAT GGGTGGTGAA GGTGAATTAC TTTCTGTAGC
AGTAGGGGTA TCAGCAGCGG TAGGAGTAGT AGTTTGTGAT TCTCCATTAT TAGTAGTACT AGTACTTGCA CTATTTGTAC
TCCTTTGACT TCCACTAGCA ATAGTATCAG CAGCGGTAGG AGTAGTAGTT TGTGATTCTC CATTATTAGT AGTACTAGTA
CTTGCACTAT TTGTACTCCT TTGACTTCCA CTAGCAATAG TATCAGCATT TGGAGCATTT GCACCTACAC TATTAGTATT
AGAACCTTCA TTTGCCATAC TTCTCCTTAT ACTCATATTA TAAGCATTGT TTATGAATGT GTTGCTATAT TTACTTTCAT
TTTTAATATT AAAGGTAACA AAA
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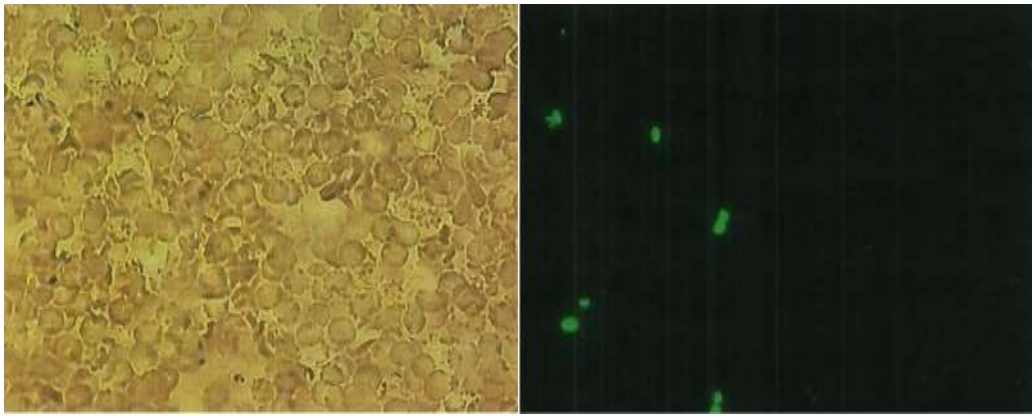
Figure 2: GFP Expression by MRA-569



/Heather Couch/
Heather Couch 16 MAY 2019
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