

***Plasmodium falciparum*, Strain D10 ACP<sub>signal</sub>-GFP**

**Catalog No. MRA-570**

**Product Description:** *Plasmodium falciparum* (*P. falciparum*), strain D10 ACP<sub>signal</sub>-GFP is a *P. falciparum*, strain D10 derivative that was created by transfection of the parent strain with a plasmid containing the acyl carrier protein (ACP) signal peptide and green fluorescent protein (GFP). *P. falciparum*, strain D10 (available as BEI Resources MRA-201) was originally isolated in Papua, New Guinea. *P. falciparum*, strain D10 ACP<sub>signal</sub>-GFP was deposited as displaying GFP fluorescence in the parasitophorous vacuole and can be utilized as a tool to study protein trafficking and plastid targeting.

**Lot<sup>1</sup>: 63792164**

**Manufacturing Date: 26OCT2015**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>2</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Genotypic Analysis</b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 720 base pairs) MSP2 PCR amplicon analysis <sup>3</sup>	Consistent with <i>P. falciparum</i> ~ 600-900 base pair amplicon	Consistent with <i>P. falciparum</i> (Figure 1) ~ 800 base pair amplicon
<b>Phenotypic Analysis</b> GFP expression <sup>4</sup>	Positive	Positive (Figure 2)
<b>Level of Parasitemia</b> Pre-freeze <sup>5</sup> Post-freeze <sup>6</sup>	Report results > 1%	4.1% 3.45%
<b>Viability (post-freeze)<sup>7</sup></b>	Growth in infected red blood cells	Growth in infected red blood cells
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>8</sup> , 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
<b>Mycoplasma Contamination</b> DNA Detection by PCR	None detected	None detected

<sup>1</sup>MRA-570 was produced by cultivation of the deposited material 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<sub>2</sub>, 5% CO<sub>2</sub>, 5% O<sub>2</sub>) and monitored for parasitemia daily for 18 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to culture to maintain 2% hematocrit.

<sup>2</sup>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.

<sup>3</sup>Primer sequences and conditions for PCR are available upon request.

<sup>4</sup>GFP expression was measured using an Olympus microscope at 100x magnification.

<sup>5</sup>Pre-freeze parasitemia was determined after 18 days post infection by microscopic counts of Giemsa-stained blood smears.

<sup>6</sup>Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.

<sup>7</sup>Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.

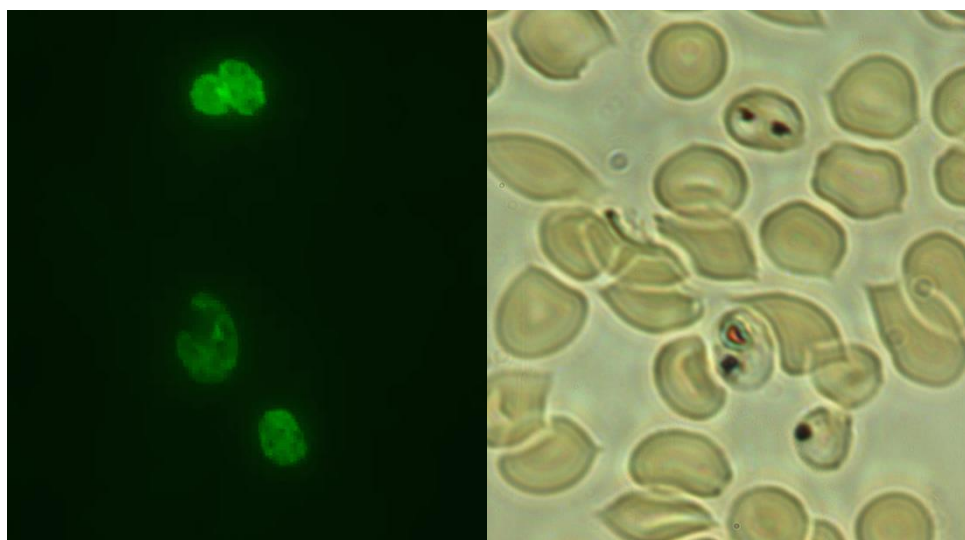
<sup>8</sup>Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

**Figure 1: MRA-570 MSP2 Sequence**

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TTTATTTTTG TTACCTTTAA TATTA AAAAT GAAAGTAAAT ATAGCAACAC ATTCATAAAC AATGCTTATA ATATGAGTAT
AAGGAGAAGT ATGGCAAATG AAGGTTCTAA TACTAATAGT GTAGGTGCAA ATGCTCCAAA TGCTGATACT ATTGCTAGTG
GAAGTCAAAG GAGTACAAAT AGTGCAAGTA CTAGTACTAC TAATAATGGA GAATCACAAA CTACTACTCC TACCGCTGCT
GATACTATTG CTAGTGGAAG TCAAAGGAGT ACAAATAGTG CAAGTACTAG TACTACTAAT AATGGAGAAT CACAAACTAC
TACTCCTACC GCTGCTGATA CCCCTACTGC TACAGAAAAGT AATTCACCTT CACCACCCAT CACTACTACA GAAAGTTCAA
GTTCTGGCAA TGCACCAAAT AAAACAGACG GTAAAGGAGA AGAGAGTGAA AAACAAAATG AATTAAATGA ATCAACTGAA
GAAGGACCCA AAGCTCCACA AGAACCTCAA ACGGCAGAAA ATGAAAATCC TGCTGCACCA GAGAATAAAG GTACAGGACA
ACATGGACAT ATGCATGGTT CTAGAAATAA TCATCCACAA AATACTTCTG ATAGTCAAAA AGAATGTACC GATGGTAACA
AAGAAAAC TG GAGCAGCA ACATCCCTCT TAAGTAACTC TAGTAATATT GCTTCAATAA ATAAATTTGT TGTTT
    
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**Figure 2: GFP Expression by MRA-570**



**Date:** 16 DEC 2015

**Signature:** 

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