

***Plasmodium falciparum*, Strain 3D7 KAHRP(+His)-GFP**

Catalog No. MRA-576

Product Description: *Plasmodium falciparum* (*P. falciparum*), strain 3D7 KAHRP(+His)-GFP is a derivative that was created by transfection of the parent 3D7 strain with a plasmid containing the knob-associated histidine-rich protein (KAHRP), the histidine rich region, and green fluorescent protein (GFP). *P. falciparum*, strain 3D7 (available as BEI Resources MRA-102) was originally isolated in the Netherlands. *P. falciparum*, strain 3D7 KAHRP(+His)-GFP was deposited as displaying fluorescence in the parasitophorous vacuole and can be utilized as a tool to study KAHRP trafficking and plastid targeting.

Lot¹: 63901361

Manufacturing Date: 24NOV2015

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy²	Blood-stage parasites present	Blood-stage parasites present
Genotypic Analysis Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 810 base pairs) MSP2 PCR amplicon analysis ³	≥ 99% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: AE001362) ~ 600-900 base pair amplicon	100% sequence identity to <i>P. falciparum</i> , strain 3D7 (GenBank: AE001362) (Figure 1) ~ 900 base pair amplicon
Phenotypic Analysis GFP expression ⁴	Positive	Positive (Figure 2)
Level of Parasitemia Pre-freeze ⁵ Post-freeze ⁶	Report results > 1%	4.65% 4.56%
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 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
Mycoplasma Contamination DNA Detection by PCR	None detected	None detected

¹MRA-576 was produced by cultivation of MRA-576 lot 3608834 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% N₂, 5% CO₂, 5% O₂) and monitored for parasitemia daily for 7 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.

²Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 6 days.

³Primer sequences and conditions for PCR are available upon request.

⁴GFP expression was measured using an Olympus microscope at 1000x magnification.

⁵Pre-freeze parasitemia was determined after 7 days post infection by microscopic counts of Giemsa-stained blood smears.

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

⁷Viability was confirmed by examination of infected erythrocytes for parasitemia at 6 days post infection.

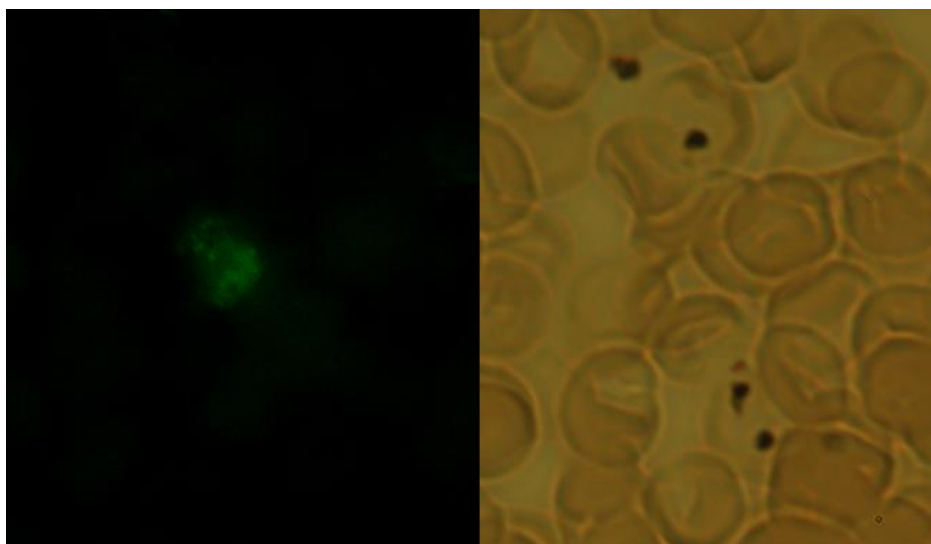
⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-576 MSP2 Sequence

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AAGGTAATTA AAACATTGTC TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTTAAA AATGAAAAGTA AATATAGCAA
CACATTCATA AACCAATGCTT ATAATATGAG TATAAGGAGA AGTATGGCAG AAAGTAAGCC TTCTACTGGT GCTGGTGGTA
GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTTC TGGTGATGGT
AATGGTGCAG ATGCTGAGGG AAGTTCAAGT ACTCCCCTA CTACCACAAC TACCAAAACCT ACCACAACCTA CCACAACCTAC
TAATGATGCA GAAGCATCTA CCAGTACCTC TTCAGAAAAT CCAAATCATA AAAATGCCGA AACAAATCCA AAAGGTAAAG
GAGAAGTTCA AGAACCAAAT CAAGCAAATA AAGAAACTCA AAATAACTCA AATGTTCAAC AAGACTCTCA AACTAAATCA
AATGTTCCAC CCACTCAAGA TGCAGACACT AAAAGTCCTA CTGCACAACC TGAACAAGCT GAAAATTCTG CTCCAACAGC
CGAACAAAAC GAATCCCCCG AATTACAATC TGCACCAGAG AATAAAGGTA CAGGACAACA TGGACATATG CATGGTTCTA
GAAATAATCA TCCACAAAAT ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG AAAACTGTGG AGCAGCAACA
TCCCTCTTAA ATAACTCTAG TAATATTGCT TCAATAAATA AATTTGTTGT TTTAATTTCA GCAACACTTG TTTTATCTTT
TGCCAT
    
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Figure 2: GFP Expression by MRA-576



Date: 31 MAY 2016

Signature: 

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