Certificate of Analysis for MRA-577

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

Catalog No. MRA-577

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 first 60 amino acids of the knob-associated histidine-rich protein [KAHRP; it lacks the histidine-rich region (residues 61 to 123)] and the green fluorescent protein (GFP). 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.

Lot[1]: 70017002 **Manufacturing Date: 16JUL2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2] Half-maximal Inhibitory Concentration (IC_{50}) by SYBR green I® drug sensitivity assay[4] Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	 Report results Report results Report results Report results Report results Report results	 5.7 ± 0.3 nM 8.4 ± 0.2 nM 26.8 ± 1.9 nM 10.7 ± 0.5 nM 37.8 ± 1.7 nM 286500 ± 26425 nM
Genotypic Analysis[2] Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 710 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943)	100% sequence identity to *P. falciparum*, strain 3D7 (GenBank: LN999943) (Figure 1)
Phenotypic Analysis GFP expression	Positive	Pending
Functional Activity by PCR Amplification[2] MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 800 base pair amplicon
Level of Parasitemia Pre-freeze[6,7] Ring-stage parasitemia Total parasitemia Post-freeze[2,8] Ring-stage parasitemia Total parasitemia	 Report results ≥ 2% Report results ≥ 1%	 4.76% 7.14% 2.58% 3.01%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2] Harpo's HTYE broth[10], 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[2] DNA Detection by PCR	None detected	None detected

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[1]MRA-577 was produced by cultivation of BEI Resources MRA-577 lot 3598560 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 10 days. Every 2 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.
[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]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: https://www.beiresources.org/Publications/MethodsinMalariaResearch.aspx].
[5]Primer sequences and conditions for PCR are available upon request.
[6]Testing completed on bulk material prior to vialing and freezing.
[7]Parasitemia was determined after 10 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 4 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 4 days post infection.
[10]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-577 MSP2 Sequence

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TTAAAAATGA AAGTAAATAT AGCAACACAT TCATAAACAA TGCTTATAAT ATGAGTATAA GGAGAAGTAT GGCAGAAAGT
AAGCCTTCTA CTGGTGCTGG TGGTAGTGCT GGTGGTAGTG CTGGTGGTAG TGCTGGTGGT AGTGCTGGTG GTAGTGCTGG
TGGTAGTGCT GGTTCTGGTG ATGGTAATGG TGCAGATGCT GAGGGAAGTT CAAGTACTCC CGCTACTACC ACAACTACCA
AAACTACCAC AACTACCACA ACTACTAATG ATGCAGAAGC ATCTACCAGT ACCTCTTCAG AAAATCCAAA TCATAAAAAT
GCCGAAACAA ATCCAAAAGG TAAAGGAGAA GTTCAAGAAC CAAATCAAGC AAATAAAGAA ACTCAAAATA ACTCAAATGT
TCAACAAGAC TCTCAAACTA AATCAAATGT TCCACCCACT CAAGATGCAG ACACTAAAAG TCCTACTGCA CAACCTGAAC
AAGCTGAAAA TTCTGCTCCA ACAGCCGAAC AAACTGAATC CCCCGAATTA CAATCTGCAC CAGAGAATAA AGGTACAGGA
CAACATGGAC ATATGCATGG TTCTAGAAAT AATCATCCAC AAAATACTTC TGATAGTCAA AAAGAATGTA CCGATGGTAA
CAAAGAAAAC TGTGGAGCAG CAACATCCCT CTTAAATAAC TCTAGTAATA TTGCTTCAAT AAATAAATT
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/Heather Couch/
Heather Couch 20 SEP 2018
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