Certificate of Analysis for MRA-1314

Plasmodium falciparum, Strain NF54::DiCre

Catalog No. MRA-1314

Product Description:

Plasmodium falciparum (P. falciparum), strain NF54::DiCre is a derivative of the NF54 strain with the rapamycin-induced dimerized Cre recombinase (DiCre) system integrated into the *pfs47* locus via CRISPR/Cas9. MRA-1314 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 every 1 to 4 days for 17 days. Every 1 to 4 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture as needed and monitored for hematocrit.

Lot: 70028492 **Manufacturing Date: 27SEP2019**

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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	10.5 ± 0.5 nM
Artemisinin	Report results	10.5 ± 0.2 nM
Quinine	Report results	51.2 ± 2.4 nM
Cycloguanil	Report results	15.4 ± 1.1 nM
Pyrimethamine	Report results	55.5 ± 3.8 nM
Sulfadoxine	Report results	410900 ± 28407 nM
Genotypic Analysis[3]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 800 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain NF54::DiCre (GenBank: QFXU01000003.1)	100% sequence identity to *P. falciparum*, strain NF54::DiCre (GenBank: QFXU01000003.1) (Figure 1)
Functional Activity by PCR Amplification[3]		
MSP2 PCR amplicon analysis	~ 600-900 base pair amplicon	~ 900 base pair amplicon
Level of Parasitemia by Giemsa Stain Microscopy		
Pre-freeze (17 days post-infection)[3]		
Ring-stage parasitemia	Report results	2.54%
Total parasitemia	≥ 2%	4.76%
Post-freeze (3 days post-infection)[1]		
Ring-stage parasitemia	Report results	0.55%
Total parasitemia	≥ 1%	1.84%
Viability (post-freeze; 3 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[4]	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

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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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: 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-1314 MSP2 Sequence

AATTAAAACA TTGTCTATTA TAAATTTCTT TATTTTTGTT ACCTTTAATA 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 AAATAAATTT GTTGTTTTAA TTTCAGCAAC ACTTGTTTTA TCTTTTGCCA
TA

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Heather Couch 19 AUG 2020
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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898