

**Plasmodium falciparum, Strain HOX**

**Catalog No. MRA-1275**

**Product Description:** *Plasmodium falciparum* (*P. falciparum*), strain HOX (High OXygen) is a derivative of the NF54 strain that was gradually adapted to proliferate in high oxygen conditions (standard tissue culture) of 5% CO<sub>2</sub> in air at 37°C. The parent *P. falciparum*, strain NF54 (available as BEI Resources MRA-1000) was isolated from a patient living near Schipol Airport, Amsterdam, who had never left the Netherlands.

**Lot<sup>1</sup>: 63802106**

**Manufacturing Date: 25NOV2015**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Giemsa Stain Microscopy<sup>2</sup></b>	Blood-stage parasites present	Blood-stage parasites present
<b>Antimalarial Susceptibility Profile (<i>in vitro</i>)</b> Half-maximal Inhibitory Concentration (IC <sub>50</sub> ) by SYBR green I <sup>®</sup> drug sensitivity assay <sup>3</sup> Chloroquine Artemisinin Quinine Cycloguanil Pyrimethamine Sulfadoxine	Report results Report results Report results Report results Report results Report results	8.5 ± 0.4 nM 26.4 ± 1.8 nM 38.2 ± 0.9 nM 5.7 ± 0.3 nM 29.1 ± 0.7 nM 469900 ± 21647 nM
<b>Genotypic Analysis</b> Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 770 base pairs)  MSP2 PCR amplicon analysis <sup>4</sup>	≥ 99% sequence identity to <i>P. falciparum</i> , strain NF54 (GenBank: AMYQ01000292.1)  ~ 600-900 base pair amplicon	100% sequence identity to <i>P. falciparum</i> , strain NF54 (GenBank: AMYQ01000292.1) (Figure 1)  ~ 900 base pair amplicon
<b>Level of Parasitemia</b> Pre-freeze <sup>5</sup> Post-freeze <sup>6</sup>	Report results > 1%	5.19% 2.06%
<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-1275 was produced by cultivation of the deposited material in fresh human erythrocytes suspended in RPMI 1640 medium without phenol red, adjusted to contain 0.5% Albumax (Gibco<sup>®</sup> 11021-037), 25 mM HEPES, 27 mM NaHCO<sub>3</sub> and 4.4 μM hypoxanthine. The culture was incubated at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> and monitored for parasitemia daily for 5 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 1-2% 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 4 days.

<sup>3</sup>A SYBR Green I<sup>®</sup> anti-malarial drug sensitivity assay in 96-well plates was used to determine IC<sub>50</sub> 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<sup>®</sup>-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.mr4.org/Publications/MethodsInMalariaResearch.aspx>.

<sup>4</sup>Primer sequences and conditions for PCR are available upon request.

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

<sup>6</sup>Post-freeze parasitemia was determined after 4 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 4 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-1275 MSP2 Sequence

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TTTTGTTACC TTTAATATTA AAAATGAAAG TAAATATAGC AACACATTCA TAAACAATGC TTATAATATG AGTATAAGGA
GAAGTATGGC AGAAAGTAAG CCTTCTACTG GTGCTGGTGG TAGTGCTGGT GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT
GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT TCTGGTGATG GTAATGGTGC AGATGCTGAG GGAAGTTCAA GTACTCCCGC
TACTACCACA ACTACCAAAA CTACCACAAC TACCACAAC ACTAATGATG CAGAAGCATC TACCAGTACC TCTTCAGAAA
ATCCAAATCA TAAAAATGCC GAAACAAATC CAAAAGGTAA AGGAGAAGTT CAAGAACCAA ATCAAGCAAA TAAAGAAACT
CAAAATAACT CAAATGTTCA ACAAGACTCT CAAACTAAAT CAAATGTTCC ACCCACTCAA GATGCAGACA CTAAGGTCC
TACTGCACAA CCTGAACAAG CTGAAAATTC TGCTCCAACA GCCGAACAAA CTGAATCCCC CGAATTACAA TCTGCACCAG
AGAATAAAGG TACAGGACAA CATGGACATA TGCATGGTTC TAGAATAAAT CATCCACAAA ATACTTCTGA TAGTCAAAAA
GAATGTACCG ATGGTAACAA AGAAAACGTG GGAGCAGCAA CATCCCTCTT AAATAACTCT AGTAATATTG CTTCAATAAA
TAAATTTGTT GTTTTAATTT CAGCAACACT TGTTTTATCT TTTGC
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Date: 19 JUL 2016

Signature:



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