

## **Certificate of Analysis for MRA-817**

### Plasmodium yoelii subsp. yoelii, Strain 17XNL:PyGFP

### Catalog No. MRA-817

**Product Description:** Plasmodium yoelii (P. yoelii) subsp. yoelii, strain 17XNL:PyGFP is a 17XNL line that is stably transfected with a vector (pL0016, available as BEI Resources MRA-785) containing the green fluorescent protein (GFP) gene. P. yoelii subsp. yoelii, strain 17XNL is a non-lethal line of the 17X strain, which was originally isolated by I. Landau from wild-caught thicket rat (Thamnomys rutilans) no. 17X, at La Maboké Field Station in Central African Republic, April 1965. P. yoelii subsp. yoelii, strain 17XNL:PyGFP is a stable line selected with pyrimethamine that constitutively expresses high levels of GFP during the complete life cycle of the parasite.

Lot<sup>1</sup>: 2166 Manufacturing Date: 20JAN2017

| TEST   | SPECIFICATIONS  | RESULTS   |  |  |
|--|---|---|--|--|
| Genotypic Analysis   |   |   |  |  |
| Sequencing of Circumsporozoite Surface Protein 1 (CSP1) gene (~ 630 base pairs)  CSP1 PCR amplicon analysis² | ≥ 99% sequence identity to  P. yoelii subsp. yoelii, strain 17XNL (GenBank: AABL01000897) ~ 900-1100 base pair amplicon | 99.7% sequence identity to P. yoelii subsp. yoelii, strain 17XNL (GenBank: AABL01000897) (Figure 1) ~ 1100 base pair amplicon |  |  |
| Phenotypic Analysis  |   |   |  |  |
| GFP expression <sup>3</sup>  | Positive  | Positive (Figure 2)   |  |  |
| Level of Parasitemia   |   |   |  |  |
| Pre-freeze <sup>4</sup>  | Report results  | 9.06%   |  |  |
| Post-freeze <sup>5</sup>   | ≥ 1′%   | 2.13%   |  |  |
| Viability (post-freeze) <sup>6</sup>   | Growth in inoculated mice   | Growth in inoculated mice   |  |  |

<sup>&</sup>lt;sup>1</sup>MRA-817 was produced by inoculation of MR-MRA-817 lot 58319583 into three ND4 Swiss Webster mice. Infection was allowed to progress for 7 days until parasitemia reached > 5%. Infected blood was collected by orbital bleeding and used to inoculate 17 ND4 Swiss Webster mice. Infection was allowed to progress until parasitemia reached > 5%. After 7 days, infected blood was collected by orbital bleeding.

#### Figure 1: MRA-817 CSP1 Sequence

| TTTATCCATT | TTACAAATTT | CAGTATCAAT | ATCCTCTAAG | GTCAAATTTT | CTGGTTGCTT | GTTTACATTT | TTTCGTTTTC |
|------------|------------|------------|------------|------------|------------|------------|------------|
| TAACTCTTAC | ACCARAACCA | CAGGTTACAC | TACATTGAGA | CCATTCCTCT | GTGAGTTGAC | TACTTATCTG | TTTAACAAAT |
| TCTAGTATTT | GTTCCGCGCT | TGGGACATAA | GAATCTTCAT | TATTATTACC | ATTATTATTG | TTATTGTTGT | TATTACCATC |
| TGGCTGTGGG | CGTGGTTGTT | GTGGTGGCTG | TTGTGGTGGC | TGTTGTGGTG | GCTGTTGTGG | TGGCTGTTGT | GGTGGTTGTT |
| GGGGTGGTTG | TTGGGGTGGT | TCTTGTGGTG | CTCCTGGCCC | TTGTGGTGCT | CCTGGCCCCT | GTGGTGCTCC | TGGTCCTTGT |
| GGTGCTCCTG | GTCCTTGTGG | TGCTCCTGGC | CCCTGTGGTG | CTCCTGGTCC | TTGTGGTGCT | CCTGGACCCT | GTGGTGCTCC |
| TGGACCCTGT | GGTGCTCCTG | GACCTTGTGG | TGCTCCTGGT | CCTTGTGGWG | CTCCTGGTCC | TTGTGGTGCT | CCTGGACCCT |
| GTGGTGCTCC | TGGTCCTTGT | GGTGCTCCTG | GCCCTTGTGG | TGCTCCTGGC | CCCTGTGGTG | CTCCTG     |            |

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<sup>&</sup>lt;sup>2</sup>Primer sequences and conditions for PCR are available upon request; see, Mauduit, M., et al. "A Role for Immune Responses against Non-CS Components in the Cross-Species Protection Induced by Immunization with Irradiated Malaria Sporozoites." PLoS One 4 (2009): e7717. PubMed: 19890387.

<sup>&</sup>lt;sup>3</sup>GFP expression was monitored by fluorescence microscopy. Fluorescence was observed in all stages of the erythrocytic life cycle.

<sup>&</sup>lt;sup>4</sup>Pre-freeze parasitemia was determined after 7 days post infection by microscopic counts of Giemsa-stained blood smears.

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

<sup>&</sup>lt;sup>6</sup>Viability was confirmed by examination of two ND4 Swiss Webster mice for parasitemia at 5 days post infection.



# **Certificate of Analysis for MRA-817**

Figure 2: GFP Expression by MRA-817

**Date:** 20 JUL 2017

Signature:

**BEI Resources Authentication** 

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