

***Plasmodium berghei*, Strain NK65 PbBlue**

Catalog No. MRA-841

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Plasmodiidae*, *Plasmodium*

Species: *Plasmodium berghei*

Strain: NK65 PbBlue

Original Source: *Plasmodium berghei* (*P. berghei*), strain NK65 PbBlue is a beta-galactosidase-expressing recombinant strain constructed using a pSE-05 targeting vector (available as BEI Resources Catalog No. MRA-842). MRA-841 is a stable line selected with pyrimethamine that features a sporozoite-specific quantifiable expression of the lacZ gene driven by a circumsporozoite protein (CSP) promoter.^{1,2}

P. berghei is a protozoan parasite that infects mammals other than humans, especially rodents. It is commonly used in rodent model studies of malaria. *P. berghei* preferentially invades reticulocytes, typically producing infections in mice that induce severe pathology.³

Material Provided:

Each vial of MRA-841 contains approximately 0.5 mL of *P. berghei*-infected mouse blood in Glycerolyte 57 solution (1:2). This item is host restricted and must be amplified in rodents. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:

MRA-841 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -80°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended (-130°C or colder). Freeze-thaw cycles should be avoided.

Growth Conditions⁴:

In vivo, Swiss Webster-ND4 mice (Alternative hosts are NMRI mice and Sprague Dawley rats).^{1,2}

Inoculation:

1. Thaw a frozen cryovial of MRA-841 in a 35°C to 37°C water bath for approximately 2 to 3 minutes. Do not allow the vial to immerse near the cap line seal while thawing.
2. Once thawed, wipe the outside of the vial with 70% ethanol before opening. Using a 1 mL syringe equipped with a 27-gauge 1/2-inch needle, remove approximately 200 µL to 300 µL from the vial.

3. Wipe the injection site of the mouse with 70% ethanol and inject the sample intraperitoneally at 100 to 150 µL per mouse (approximately 3 mice for most applications).

Monitoring parasitemia:

1. Starting 3 days post-inoculation, monitor the growth of parasites by tail vein bleed sampling and Giemsa-stained thin blood smear microscopy at 1- to 2-day intervals.
2. Passage the strain when the infection is at or near the first peak of parasitemia (> 5%). This will normally occur within one week of inoculation.

$$\% \text{ parasitemia} = (\text{Infected RBC} / \text{Total RBC}) \times 100$$

Passaging:

1. Anesthetize infected mice by CO₂/O₂ inhalation. Collect the blood by orbital bleeding or from the tail vein into 25 mL of cold 1× PBS-heparin anticoagulant solution (please refer to Appendix I for preparation instructions).
2. Inject the sample into each of the uninfected mice (approximately 10 mice) as described in Inoculation step #3 above.
3. Monitor parasitemia as described above and passage as needed.

Please refer to Appendix I for cryopreservation instructions.

Note: Do not directly inject freshly thawed parasites from cryopreserved stocks by the intravenous (IV) route, as these samples contain cryoprotectant, anticoagulant and may contain traces of lysed or coagulated red blood cells. Direct IV inoculation from cryopreserved stock may result in pulmonary embolism or shock in mice.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Plasmodium berghei*, Strain NK65 PbBlue, MRA-841, contributed by Photini Sinnis.”

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

All blood cultures should be handled with appropriate safety precautions necessary for the handling of bloodborne pathogens. Personnel must be trained in accordance with their institutional policy regarding bloodborne pathogens.

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APPENDIX I: CRYOPRESERVATION

Note: Only immature blood-stage parasites (rings) are viable by this method. Parasitemia of 1% or higher of ring-stage parasites is recommended for cryopreservation. All steps should be carried out in a biosafety cabinet under proper airflow.

1. Prepare a 1× PBS-heparin anticoagulant solution using sterile 1× PBS (pH ~ 7.2) without calcium or magnesium (ATCC® 30-2200™) adjusted to contain 30 Units per milliliter of sterile heparin.
2. Harvest parasitized mouse blood into 25× volume ice-cold sterile 1× PBS-heparin anticoagulant solution and place on ice.
3. Centrifuge the diluted blood culture at 1000 × g for 5 minutes at 4°C.
4. Aspirate the supernatant carefully. Measure the volume of packed red blood cells using centrifuge tube graduations or standard volume controls.
5. To the volume of packed red blood cells, add dropwise one volume of cold (4°C) Glycerolyte 57 solution (Fenwal, Catalog No. 4A7831, or equivalent). Let stand for 5 minutes at room temperature.
6. Add dropwise an additional volume of cold Glycerolyte 57 solution to the pellet. Mix well and aliquot 0.5 mL into 1.5 mL sterile cryopreservation vials.
7. Place the vials in a controlled-rate freezing unit. From room temperature, cool the vials at -1°C per minute to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per minute through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1 to 2 days and then plunge vials into liquid nitrogen.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

References:

1. Sinnis, P., Personal Communication.
2. Engelmann, S., P. Sinnis and K. Matuschewskil. "Transgenic *Plasmodium berghei* Sporozoites Expressing Beta-Galactosidase for Quantification of Sporozoite Transmission." *Mol. Biochem. Parasitol.* 146 (2006): 30-37. PubMed: 16316690.
3. Lin, J., et al. "Identification of Disease-Related Genes in *Plasmodium berghei* by Network Module Analysis." *BMC Microbiol.* 23 (2023). PubMed: 37735351.
4. Peters, W. and B. L. Robinson (1999) "Chapter 92 -- Malaria." In *Handbook of Animal Models of Infection*. Eds. O. Zak and M. Sande, Academic Press: London, pp. 757-773.

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