

***Plasmodium berghei*, Strain ANKA****Catalog No. MRA-311****For research use only. Not for human use.****Contributor:**

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**Manufacturer:**

BEI Resources

**Product Description:**

Protozoa Classification: *Plasmodiidae*, *Plasmodium*

Species: *Plasmodium berghei*

Strain: ANKA

Original Source: *Plasmodium berghei* (*P. berghei*), strain ANKA was isolated in July 1965 from *Anopheles durenii millecampsii* mosquitoes collected in the River Kasapa, Democratic Republic of Congo.<sup>1</sup>

Comments: MRA-311 was authenticated by the contributor and deposited to MR4 in BALB/c mouse blood in 2002. The whole genome sequence of *P. berghei*, strain ANKA is available (GenBank: [CAA100000000](https://www.ncbi.nlm.nih.gov/nuccore/CAA100000000)).<sup>2</sup>

*P. berghei* is a protozoan parasite that infects mammals other than humans, especially rodents. It is commonly used in rodent model studies of malaria.<sup>2</sup>

**Material Provided:**

Each vial of MRA-311 contains approximately 0.5 mL of *P. berghei* infected mouse blood in cryopreservative. Please see Appendix I for cryopreservation instructions.

**Packaging/Storage:**

MRA-311 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 Conditions<sup>3</sup>:**

Thaw a frozen cryovial of MRA-311 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. Once thawed, wipe the outside of the vial with 70% to 100% ethanol before opening. Using a 27 gauge 1/2 inch needle, inject thawed parasites into mice via intraperitoneal injection (IP) at 50 µL to 100 µL per mouse. Monitor growth of parasites by tail vein bleed sampling and Giemsa-stained thin blood smear microscopy daily, starting on day 3 post-inoculation. Harvest and/or serially passage infected blood to naive mice when parasitemia reaches 1% to 5%, before significant signs of infection appear. Some strains of mice

may require dietary or drug pretreatment protocols for successful infection as *P. berghei* strains have a strong predilection for invasion of reticulocytes. To maintain the parasite strain *in vivo*, passage infected blood from a donor mouse to a recipient mouse using intraperitoneal or intravenous injection (IV) of an appropriately diluted inoculum as required for the study.

Note: Do not directly inject freshly thawed parasites from cryopreserved stocks by the 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 ANKA, MRA-311, contributed by Thomas F. McCutchan."

**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. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see [www.cdc.gov/biosafety/publications/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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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**References:**

1. Ramiro, R. S., S. E. Reece and D. J. Obbard. "Molecular Evolution and Phylogenetics of Rodent Malaria

Parasites." *BMC Evol. Biol.* 12 (2012): 219. PubMed: 23151308.

2. Hall, N., et al. "A Comprehensive Survey of the *Plasmodium* Life Cycle by Genomic, Transcriptomic, and Proteomic Analyses." *Science* 307 (2005): 82-86. PubMed: 15637271.

3. 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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**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 air flow.

1. Harvest parasitized mouse blood into 25 x volume ice cold sterile PBS (pH ~ 7.2) containing an appropriate concentration of anticoagulant (e.g. Heparin at 10 units/mL) and place on ice.
2. Centrifuge the diluted blood culture at 1000 x g for 5 minutes at 4°C.
3. Aspirate the supernatant carefully. Measure the volume of packed red blood cells using centrifuge tube graduations or standard volume controls.
4. To the volume of packed red blood cells, add dropwise one volume of cold (4°C) Glycerolyte 57 solution. Let stand for 5 minutes at room temperature.
5. 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.
6. Place the vials in a controlled-rate freezing unit. From room temperature, cool the vials at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min 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 24 to 48 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).