Product Information Sheet for MRA-905

Plasmodium berghei, Strain NK65 RedStar

Catalog No. MRA-905

For research use only. Not for human use.

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Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Plasmodiidae, Plasmodium*
Species: *Plasmodium berghei*
Strain: NK65 RedStar
Original Source: *Plasmodium berghei (P. berghei)*, strain NK65 RedStar is a clone of *P. berghei*, strain NK65.[1,2] *P. berghei*, strain NK65 was isolated in April 1964 from *Anopheles dureni millecampsi* mosquitoes collected in the River Kisanga, Democratic Republic of Congo.[3]
Comment: *P. berghei*, strain NK65 RedStar was deposited as a pyrimethamine-resistant parasite that stably expresses a red fluorescent protein (RFP), RedStar, during sporozoite stages.[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.[4]

Material Provided:
Each vial of MRA-905 contains approximately 0.5 mL of *P. berghei*, strain NK65 RedStar infected mouse blood in Glycerolyte 57 solution (1:2). Please see Appendix I for cryopreservation instructions.

Packaging/Storage:
MRA-905 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[5]:
Thaw a frozen cryovial of MRA-905 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. To maintain the parasite strain *in vivo*, passage infected blood from a donor mouse to a recipient mouse using intraperitoneal or intravenous (IV) injection 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 NK65 RedStar, MRA-905, contributed by Ute Frevert."

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.

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.

Disclaimers:
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References:

1. Frevert, U., Personal Communication.
2. Frevert, U., et al. "Intravital Obervation of *Plasmodium berghei* Sporozoite Infection of the Liver." PLoS Biol. 3 (2005): e192. PubMed: 15901208.
3. 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.
4. 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.
5. 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.
6. Engelmann, S., P. Sinnis and K. Matuschewski. "Transgenic *Plasmodium berghei* Sporozoites Expressing Beta-Galactosidase for Quantification of Sporozoite Transmission." Mol. Biochem. Parasitol. 146 (2006): 30-37. PubMed: 16316690.
7. Knop, M., et al. "Improved Version of the Red Fluorescent Protein (drFP583/DsRed/RFP)." Biotechniques 33 (2002): 592-602. PubMed: 12238769.

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 × 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 × 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).

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